

Science

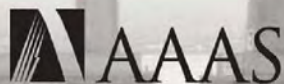
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AAAS ANNUAL MEETING

18–22 February, 2010

San Diego, California



Go to
Page 876
for Details

High Efficiency Gene Transfer into Stem Cells

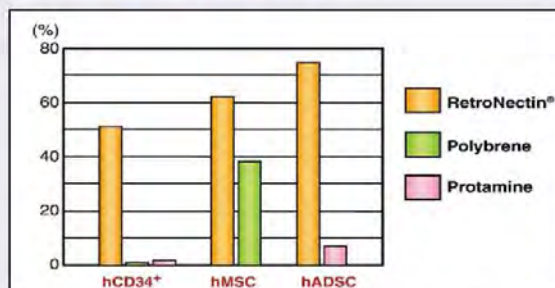
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Recombinant Human Fibronectin Fragment

Use of RetroNectin®-based gene transduction protocols dramatically enhances the efficiency of retrovirus or lentivirus-mediated gene transfer into mammalian cells. RetroNectin® is a recombinant polypeptide consisting of three functional domains derived from the human fibronectin. RetroNectin®'s enhancement of gene transduction efficiency is hypothetically due to co-localization of retroviral particles and target cells on the RetroNectin® molecule. Recent experiments demonstrate RetroNectin®'s efficacy on stem cells.

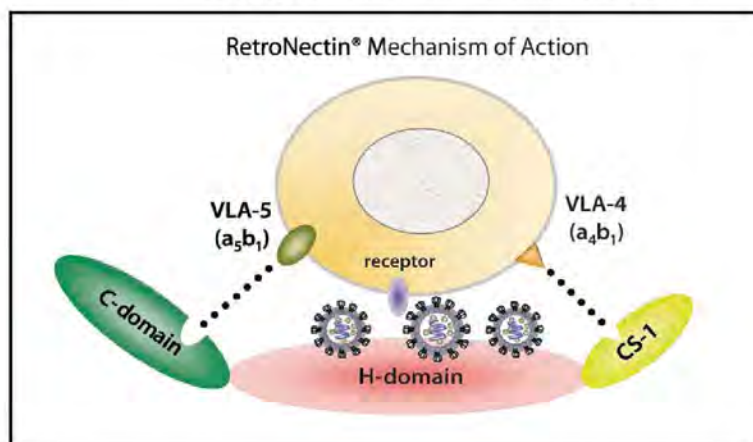
- **Highly Efficient Retroviral or Lentiviral Gene Transfer** into Stem Cells*
- **No Polybrene Required** for Transduction
- **Facilitates development of innovative protocols** for proliferation of cells carrying a transferred gene, maintaining the function of the cells, and increasing transplant efficiency

* carrying VLA-4 and/or VLA-5 cell surface receptors



Comparison of retrovirus-mediated gene transduction efficiency in various methods into human stem cells.

RetroNectin® enables and enhances retrovirus-mediated gene transfer into stem cells which express lower levels of GALV envelope receptor, such as hCD34+ or hADSC



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Annexin-V
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Apolipoprotein E2
Apolipoprotein E3
Apolipoprotein E4
APRIL
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Aurora B
BAFF
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BD-1
BD-2
BD-3
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Endostatin
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Eotaxin / CCL11
Eotaxin-2
Eotaxin-3 (TSC)
EPHB2
EPHB4
Epigen
Epregrulin
Eptilbatide
Erk-2
Erythropoietin (EPO)
Exodus-2
Fas Ligand
Fas Receptor
FGF-1 (acidic)
FGF-2 (basic)
FGF-4
FGF-5
FGF-6
FGF-7 / KGF
FGF-8
FGF-9
FGF-10
FGF-16
FGF-17
FGF-18
FGF-19
FGF-20
sFGFR-1 (IIIC) / Fc Chimera
sFGFR-2 (IIIC) / Fc Chimera
sFGFR-3 / Fc Chimera
sFGFR-4 / Fc Chimera
sFlt-1 (native)
sFlt-1 (D3)
sFlt-1 (D4)
sFlt-1 (D5)
sFlt-1 (D7)
Flt3-Ligand
sFlt-4
sFlt-4 / Fc Chimera
Follistatin
FSH
Fractalkine / CX3C
G-CSF
 α -Galactosidase A
Galectin-1

Galectin-3
Gastrointestinal CA
GCP-2
GDF-3
GDF-9
GDF-11
GDNF
GLP-1
Glucagon
GM-CSF
Goserelin
GPBB
Granzyme B
GRO α
GRO β
GRO γ
GRO/MGSA
Growth Hormone
Growth Hormone BP
GST-p21/WAF-1
HB-EGF
HCC-1
HGF
Histidyl-IRNA synthetase
Histrelin
HRG1- β 1
I-309
I-TAC
IFN- α
IFN- α A
IFN- α 2a
IFN- α 2b
IFN- β
IFN- γ
IFN-Omega
IGF-I
IGF-II
proIGF-II
IGFBP-1
IGFBP-2
IGFBP-3
IGFBP-4
IGFBP-5
IGFBP-6
IGFBP-7
IL-1 α
IL-1 β
IL-2
IL-3
IL-4
sIL-4 Receptor
IL-5
IL-6
sIL-6 Receptor
IL-7
IL-8 (72 a.a.)
IL-8 (77 a.a.)
IL-9
IL-10
IL-11
IL-12
IL-13
IL-13 analog
IL-15
IL-16 (121 a.a.)
IL-16 (130 a.a.)
IL-17
IL-17B
IL-17D

IL-17E
IL-17F
IL-19
IL-20
IL-21
IL-22
IL-31
Insulin
IP-10
JE
JNK2a1
JNK2a2
KC / CXCL1
KGF
L-asparaginase
LAG-1
LALF Peptide
LAR-PTP
LBP
LC-1
LD-78 β
LDH
LEC / NCC-4
Leptin
LIGHT
LIX
LKM
LL-37
Lungkine / CXCL15
Lymphotactin
sLYVE-1
M-CSF
MCP-1 (MCAF)
MCP-2
MCP-3
MCP-4
MCP-5
MDC (67 a.a.)
MDC (69 a.a.)
MDH
MEC
Mek-1
MIA
Midkine
MIG / CXCL9
MIP-1 α / CCL3
MIP-1 β / CCL4
MIP-3 / CCL23
MIP-3 α / CCL20
MIP-3 β / CCL19
MIP-4 (PARC) / CCL18
MIP-5 / CCL15
MMP-3
MMP-7
MMP-13
Myostatin
Nanog
NAP-2
Neurturin
NFAT-1
 β -NGF
NOGGIN
NOV
NP-1
NT-1/BCSF-3
NT-3
NT-4
Ocreotide
Oncostatin M
Osteoprotegerin (OPG)
OTOR
Oxytocin
p38- α
PAI-1
Parathyroid Hormone
PDGF-AA
PDGF-AB
PDGF-BB
PDGF-CC
Persephin
PF-4
PIGF-1

PIGF-2
PKA α -subunit
PKC- α
PKC- γ
Pleiotrophin
PLGF-1
Polymyxin B (PMB)
PRAS40
PRL-1
PRL-2
PRL-3
Prokineticin-2
Prolactin
Protirelin
PTHrP
PTP1B
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PTP-MEG2
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sRANK
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RELM- α
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RELM- β
Resistin
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RPTP μ
SCF
SCGF- α
SCGF- β
SDF-1 α
SDF-1 β
Secretin
SF20
SHP-2
STAT1
c-Src
TACI
TARC
TC-PTP
TECK
TFF2
TGF- α
TGF- β 1
TGF- β 2
TGF- β 3
Thymosin α 1
sTIE-1/Fc Chimera
sTIE-2/Fc Chimera
TL-1A
TNF- α
TNF- β
sTNF-receptor Type I
sTNF-receptor Type II
TPO
sTRAIL R-1 (DR4)
sTRAIL R-2 (DR5)
TRAIL/Apo2L
TSG
TSH
TSLP
TWEAK
TWEAK Receptor
Urokinase
EG-VEGF
VEGF121
VEGF145
VEGF165
VEGF-C
VEGF-C 1125
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EDITORIAL

- 771 Development and Climate Change
Rosina M. Bierbaum and Robert B. Zoellick

NEWS OF THE WEEK

- 778 Helium-3 Shortage Could Put Freeze on Low-Temperature Physics
- 779 Peer Review Not Popular at Homeland Security
- 780 CIRM Awards Seek to Move Cell Therapies to the Clinic
- 780 Court Orders Stanford Expert to Surrender Manuscript
- 781 From *Science's* Online Daily News Site
- 782 Developing Countries to Get Some H1N1 Vaccine—But When?
- 783 When Counting Jobs Isn't Enough
- 783 From the *Science* Policy Blog

NEWS FOCUS

- 784 ORIGINS
On the Origin of Religion
>> Science Podcast
- 788 20 YEARS AFTER THE WALL
Aufbau Ost:
Max Planck's East German Experiment
Why So Few East German Directors?
- 792 Big Dreams Come True
>> Science Careers section p. 765
- 794 No Genome Left Behind

LETTERS

- 797 Space Goals Require
Worldwide Participation
W. E. Howard III
- Biobanks: Questioning Distinctions
M. G. Hansson and K. J. Maschke
- Biobanks: Too Long to Wait for Consent
K. B. Brothers and E. W. Clayton

- Biobanks: Oversight Offers Protection
K. Hens et al.
- Response
D. Gurwitz et al.
- Research for All Science Teachers
J. Dickey

798 CORRECTIONS AND CLARIFICATIONS

BOOKS ET AL.

- 800 Stephen Jay Gould and the Politics of Evolution
D. F. Prindle, reviewed by V. B. Smocovitis
- 801 Evolution of the End of *Origin*
R. W. D. Nickalls

EDUCATION FORUMS

- 802 Building on No Child Left Behind
E. A. Hanushek
- 803 Moving Past No Child Left Behind
D. Koretz

PERSPECTIVES

- 805 A Comeback for Gene Therapy
L. Naldini
>> Research Article p. 818
- 806 Biodiversity and Climate Change
K. J. Willis and S. A. Bhagwat
- 808 Evolution of Animal Pollination
J. Ollerton and E. Coulthard
>> Report p. 840
- 809 Capturing the Complexities of Molecule-Surface Interactions
E. Hasselbrink
>> Reports pp. 829 and 832
- 810 Peatland Response to Global Change
N. B. Dise

CONTENTS continued >>



page 784



page 788



COVER

The Coronado Bridge links the cities of San Diego and Coronado, California. The theme of the AAAS Annual Meeting in San Diego, 18 to 22 February 2010, acknowledges that the relevance of science, technology, and engineering as well as scientific literacy to the well-being of society is more profound than ever. The preliminary program begins on page 876.

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DEPARTMENTS

- 767 This Week in *Science*
- 773 Editors' Choice
- 774 *Science* Staff
- 777 Random Samples
- 875 New Products
- 876 AAAS Meeting Program
- 886 *Science* Careers

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REVIEW

- 812 **Einstein's Theory of Gravity and the Problem of Missing Mass**
P. G. Ferreira and G. D. Starkman

BREVIA

- 816 **Population Structure Mediates Sexual Conflict in Water Striders**
O. T. Eldakar et al.
When water striders can disperse among groups, females select for reduced male aggression.
- 817 **Genotype Analysis Identifies the Cause of the "Royal Disease"**
E. I. Rogaev et al.
DNA from historical specimens reveals the mutation causing the hemophilia that afflicted the royal families of Europe.

RESEARCH ARTICLE

- 818 **Hematopoietic Stem Cell Gene Therapy with a Lentiviral Vector in X-Linked Adrenoleukodystrophy**
N. Cartier et al.
Lentiviral-mediated gene therapy of hematopoietic stem cells delays disease progression in patients with a fatal brain disorder.
>> Perspective p. 805

REPORTS

- 823 **Three-Color Entanglement**
A. S. Coelho et al.
Three bright light beams of different colors can be entangled.
- 826 **Electronic Structure Controls Reactivity of Size-Selected Pd Clusters Adsorbed on TiO₂ Surfaces**
W. E. Kaden et al.
The activity of these model catalysts for carbon monoxide oxidation reflects changes in cluster electronic structure.
- 829 **Dynamical Steering and Electronic Excitation in NO Scattering from a Gold Surface**
N. Shenvi et al.
Theory accounts for the complex ways in which vibrations and rotations of nitric oxide molecules affect scattering from a surface.
>> Perspective p. 809
- 832 **Chemically Accurate Simulation of a Prototypical Surface Reaction: H₂ Dissociation on Cu(111)**
C. Díaz et al.
The use of a fitting parameter produces a much-improved potential energy surface for describing a surface reaction.
>> Perspective p. 809

- 835 **Shifts in Lake N:P Stoichiometry and Nutrient Limitation Driven by Atmospheric Nitrogen Deposition**
J. J. Elser et al.
Deposition of anthropogenically derived nitrogen can cause phosphorus to become the limiting nutrient of lake phytoplankton.
- 837 **Abiotic Gas Formation Drives Nitrogen Loss from a Desert Ecosystem**
C. K. McCalley and J. P. Sparks
In the Mojave Desert, high surface temperatures cause large amounts of nitrogen to be lost from the soil.
- 840 **A Probable Pollination Mode Before Angiosperms: Eurasian, Long-Proboscis Scorpionflies**
D. Ren et al.
Prior to the coevolution of angiosperms and pollinating insects, scorpionflies may have been pollinating gymnosperms.
>> Perspective p. 808
- 847 **Polymorphic Butterfly Reveals the Missing Link in Ecological Speciation**
N. L. Chamberlain et al.
Mate selection based on preferences for polymorphic wing color patterns is generating reproductive isolation.
- 850 **A Type I-Secreted, Sulfated Peptide Triggers XA21-Mediated Innate Immunity**
S.-W. Lee
The bacterial trigger for a rice innate immune response is identified.
- 853 **Small-Molecule Activators of a Proenzyme**
D. W. Wolan et al.
Small molecules that promote a procaspase conformation susceptible to activation by proteolysis have been identified.
- 858 **High Diversity of the Viral Community from an Antarctic Lake**
A. López-Bueno et al.
Virus populations in polar freshwater lakes show marked shifts in composition during ice melt.
- 861 **Viral Glycosphingolipids Induce Lytic Infection and Cell Death in Marine Phytoplankton**
A. Vardi et al.
A specific virus encodes membrane components that broadcast cell death and population demise of its coccolithophore host.
- 865 **Genome Sequence, Comparative Analysis, and Population Genetics of the Domestic Horse**
C. M. Wade et al.
The horse genome reveals an evolutionary new centromere and conserved chromosomal sequences relative to other mammals.



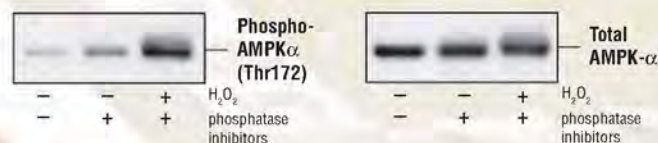
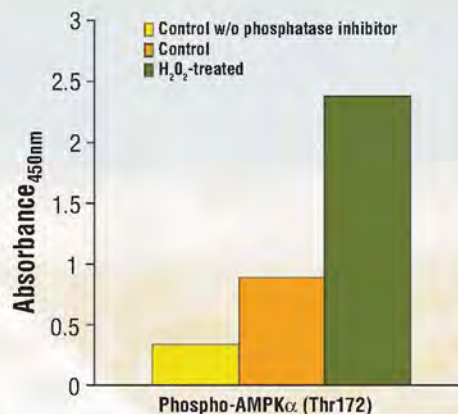
pages 805 & 818



page 858

- 867 **MafB/c-Maf Deficiency Enables Self-Renewal of Differentiated Functional Macrophages**
A. Aziz et al.
The absence of two transcription factors allows long-term propagation of a differentiated immune cell population that is nontumorigenic.
- 871 **A Single Peptide-MHC Complex Positively Selects a Diverse and Specific CD8 T Cell Repertoire**
B. Wang et al.
Positive selection by a single peptide-MHC complex imparts exquisite specificity to developing T cells.

CONTENTS continued >>



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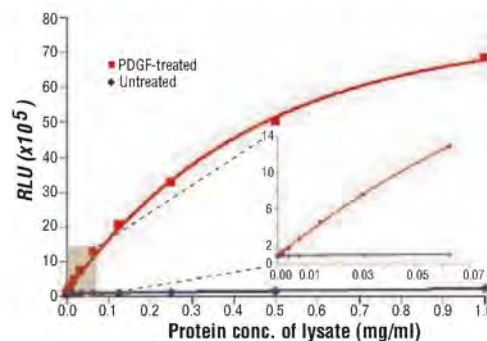
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Above: Treatment of C2C12 cells with H₂O₂ stimulates phosphorylation of AMPKα at Thr172, detected by the PathScan® Phospho-AMPKα (Thr172) Sandwich ELISA Kit #7959. The absorbance readings at 450 nm are shown in the top figure, while the corresponding western blots using Phospho-AMPKα (Thr172) (D79.5E) XP™ Rabbit mAb #4188 (left panel) or AMPKα (23A3) Rabbit mAb #2603 (right panel) are shown in the bottom figure.

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R. Drmanac et al.

A low-cost sequencing technique advances us closer to the goal of the \$1000 human genome.
10.1126/science.1181498

Bacterial Community Variation in Human Body Habitats Across Space and Time
E. K. Costello et al.

The composition of microbial communities on the human body is primarily determined by their location.
10.1126/science.1177486

Structure of the LKB1-STRAD-MO25 Complex Reveals an Allosteric Mechanism of Kinase Activation
E. Zehiraj et al.

A "pseudokinase" activates the LKB1 tumor suppressor protein without catalyzing phosphorylation.
10.1126/science.1178377

An Unusually Fast-Evolving Supernova
D. Poznanski et al.

The distinctive properties of this supernova suggest that it is of a kind predicted by theory but not previously observed.
10.1126/science.1181709
>> *Science Podcast*

Bridging the Montreal-Kyoto Gap
J. Cohen et al.
10.1126/science.1176958

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RESEARCH ARTICLE: The Rfx4 Transcription Factor Modulates Shh Signaling by Regional Control of Ciliogenesis

A. M. Ashique et al.

Rfx4 regulates the formation of primary cilia, thereby playing a crucial role in central nervous system development.

RESEARCH ARTICLE: Activation of a Bacterial Virulence Protein by the GTPase RhoA

M. Christen et al.

An enzyme essential for the virulence of *Salmonella* in mammals is activated by the GTP-bound form of RhoA.

PERSPECTIVE: In with the TRP Channels—Intracellular Functions for TRPM1 and TRPM2

S. Patel and R. Docampo

Cationic channels of the TRP melastatin family are involved in pigmentation and oxidative stress responses.

MEETING REPORT: The Hippo Tumor Suppressor Pathway—A Brainstorming Workshop

G. Blandino et al.

Researchers of flies and mammals met to discuss components of the Hippo signaling pathway.

E-LETTER: Innovators Award

Science Signaling Editors

Science Signaling authors E. S. Emamian and A. Abdi received an Innovators Award from the New Jersey Inventors Hall of Fame.

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Eastern European scientists have more chances to benefit from international collaborations.

On Going Home: Succeeding in Science in Eastern Europe

E. Pain

Three early-career scientists discuss returning to their countries after spending time abroad.
>> *News Focus* stories pp. 788 and 792

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COMMENTARY: Translational Medicine—An Engine of Change for Bringing New Technology to Community Health

C.-P. Milne and K. Kaitin

A patient-centered focus in R&D should lead to improvements in medical care.

COMMENTARY: A Translational Research Niche for SBIR Grants

K. D. Handelsman

Funding from the Small Business Innovation Research (SBIR) program can be part of the health care solution.

RESEARCH ARTICLE: Common Defects of ABCG2, a High-Capacity Urate Exporter, Cause Gout

H. Matsuo et al.

Common mutations cause gout in a Japanese population.

RESEARCH ARTICLE: Tetracyclines That Promote SMN2 Exon 7 Splicing as Therapeutics for Spinal Muscular Atrophy

M. Hastings et al.

A new class of compounds may be useful for the treatment of a leading genetic cause of infant mortality.

RESEARCH ARTICLE: Serum Amyloid P Inhibits Fibrosis Through FcγR-Dependent Monocyte-Macrophage Regulation in Vivo

A. Castano et al.

Therapeutic administration of human serum amyloid P inhibits fibrosis progression in the mouse kidney.

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A History of Beginnings

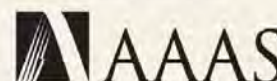
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Science Policy News and Analysis

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A Horse Is a Horse, of Course

The history of horse domestication is closely tied to the history of the human society. **Wade *et al.*** (p. 865) report on the sequencing and provide a single nucleotide polymorphism map of the horse (*Equus caballus*) genome. Horses are a member of the order perissodactyla (odd-toed animals with hooves). The analysis reveals an evolutionarily new centromere on equine chromosome 11 that displays properties of an immature but fully functioning centromere and is devoid of centromeric satellite sequence. The findings clarify the nature of genetic diversity within and across horse breeds and suggest that the horse was domesticated from a relatively large number of females, but few males.

Cluster Electronics and Catalysis

Many practical catalysts consist of small metal clusters on oxide supports, and the activity of these clusters usually varies with their size. In order to sort out some of the competing effects that lead to such variations, **Kaden *et al.*** (p. 826) size-selected palladium clusters (from single atoms to clusters up to 25 atoms) and deposited them on a crystal face of the rutile phase of titanium dioxide. X-ray photoemission studies and temperature-programmed reaction measurements showed that the activity of these model catalysts for CO oxidation was related to the electronic energy, which was reflected in the Pd 3d

electron binding energy. Ion-scattering studies showed that the clusters formed flat single- or double-layer islands.

Simulating Surfaces

Although modern computational chemistry can often match or even exceed experimental accuracy in modeling gas phase reactions, the surface-bound processes involved in most practical catalysis pose a substantially greater challenge to theory (see the Perspective by **Hasselbrink**). **Díaz *et al.*** (p. 832) show that a modification to standard density functional methods can predict reaction barrier heights to within 1 kilocalorie per mole for the widely studied dissociative adsorption of dihydro-

gen on copper. In a complementary study, **Shenvi *et al.*** (p. 829) apply an efficient algorithmic framework to model transitions among multiple electronic states at a metal surface and successfully account for the complex dependence of nitric oxide scattering on the small molecule's vibrations and rotations.

Missing Mass Explained?

The motion and distribution of galaxies and clusters of galaxies within the universe suggest that there is far more matter than can be seen directly through telescopes. Alternatively, perhaps our understanding of gravity is flawed, leading to a mismatch between the gravitational field inferred from the observed mass distribution in the universe and the observed gravitational field. **Ferreira and Starkman** (p. 812) review the viability of modifying theories of gravity to solve the problem of missing mass. It emerges that theories of modified gravity remain viable but have become more complex, involving gravitating invisible elements. However, what you see is still not what you get.

Nitrogen Overload

The cycling of essential nutrients in terrestrial ecosystems has been altered by human activities. **Elser *et al.*** (p. 835) report a comparative analysis of lakes in Norway, Sweden, and in the United States that suggests that this is also true in aquatic ecosystems such as lakes. Deposition of anthropogenically derived atmospheric nitrogen controls whether N or P is growth-limiting for phytoplankton. Under elevated conditions of atmospheric N inputs, lake phytoplankton become consistently P-limited because the N:P ratio is strongly distorted. This is in contrast to conditions of low N deposition when lake phytoplankton are N-limited. These effects are even observed in remote lakes, demonstrating the indirect yet wide-ranging effects of humans on global food webs.



Entangling Rainbows

Quantum mechanical entanglement is at the heart of quantum information processing. In the

Continued on page 769

EXTRAORDINARY REWARDS

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future, practical systems will contain a network of quantum components, possibly operating at different frequencies. **Coelho et al.** (p. 823, published online 17 September) present a technique that can entangle light beams of three different frequencies. The ability to swap entanglement between different light fields should prove useful in advanced quantum information protocols on systems comprising different operating frequencies.

Long-Lost Pollinators

The rise of angiosperms in the Early Cretaceous (~140 million years ago) was accompanied by coevolution of a variety of insects, including flies, bees, and wasps required for pollination. **Ren et al.** (p. 840; see the Perspective by **Ollerton and Coulthard**) show that three families of scorpionflies had already evolved specialized mouth parts for feeding on the nectar of gymnosperms, as early as the Middle Jurassic (~170 million years ago). The diversity and specialization of these insects and related plant structures suggests that they were also involved in pollination. These families died out later in the Cretaceous as angiosperms began to dominate.

Butterfly Apartheid

Heliconius butterflies show differences in mimetic color patterns across geographic races associated with patterns of assortative mating, suggesting that ecological speciation may be ongoing. **Chamberlain et al.** (p. 847) demonstrate assortative mating on the basis of color pattern mimicry that generates reproductive isolation between *Heliconius cydno* species and subspecies within polymorphic populations in Ecuador. Furthermore, it appears that these traits are controlled by a single gene that affects color pigment in wing pattern formation and vision. Thus, these butterflies are indeed in the early stages of reproductive isolation that is being driven by an ecological trait, allowing observation of an incipient speciation event.

Slowing Brain Disease with Gene Therapy

X-linked adrenoleukodystrophy (ALD), the hereditary brain demyelinating disorder that was featured in the movie "*Lorenzo's Oil*," is typically treated by transplantation of bone marrow from matched donors. This treatment slows progression of the disease by introducing cells that differentiate into myelin-producing cells. **Cartier et al.** (p. 818; see Perspective by **Naldini**) tested an alternative gene therapy-based approach in two young patients without matched donors. A lentiviral vector was used to introduce a wild-type copy of the ALD gene into the patients' hematopoietic stem cells *ex vivo*. The modified cells were then infused back into the patients. Expression of the transferred gene was still detectable in the patients' blood cells 2 years later, and both patients showed neurological improvement and a delay in disease progression comparable to that seen with bone marrow transplants.



Bacterial Trigger of Plant Protection

Innate immunity can be rapidly activated to defend a host plant against a microbial pathogen. The rice protein XA21, which is thought to be a cell surface-located receptor with a kinase domain, activates the plant's defenses in response to infection by certain strains of *Xanthomonas* bacteria. **Lee et al.** (p. 850) have now identified the bacterial gene that encodes the protein, AvrXA21, to which the plant receptor XA21 responds. The 194-amino acid protein needs to be secreted and sulfated to trigger the rice plant defense responses. Similarities exist between the receptor XA21 and other immune response receptors in both plants and animals.

The Death of Cocco

Emiliania huxleyi is a coccolithophore, a class of unicellular phytoplankton that forms vast blooms mediating the oceanic carbon cycle through shedding of its calcium carbonate scales. *E. huxleyi* is routinely infected and killed by lytic viruses that can abruptly halt a bloom. **Vardi et al.** (p. 861) have found that in *E. huxleyi* strains that are sensitive or resistant to infection, a sphingolipid-based "arms race" appears to regulate cell fate during host-virus interactions. The lipid also serves as a biomarker for active infection that may help to quantify the role and activity of viruses and virus-mediated processes in the oceans. This information will help in assessing the biogeochemical impact of these plankton species.

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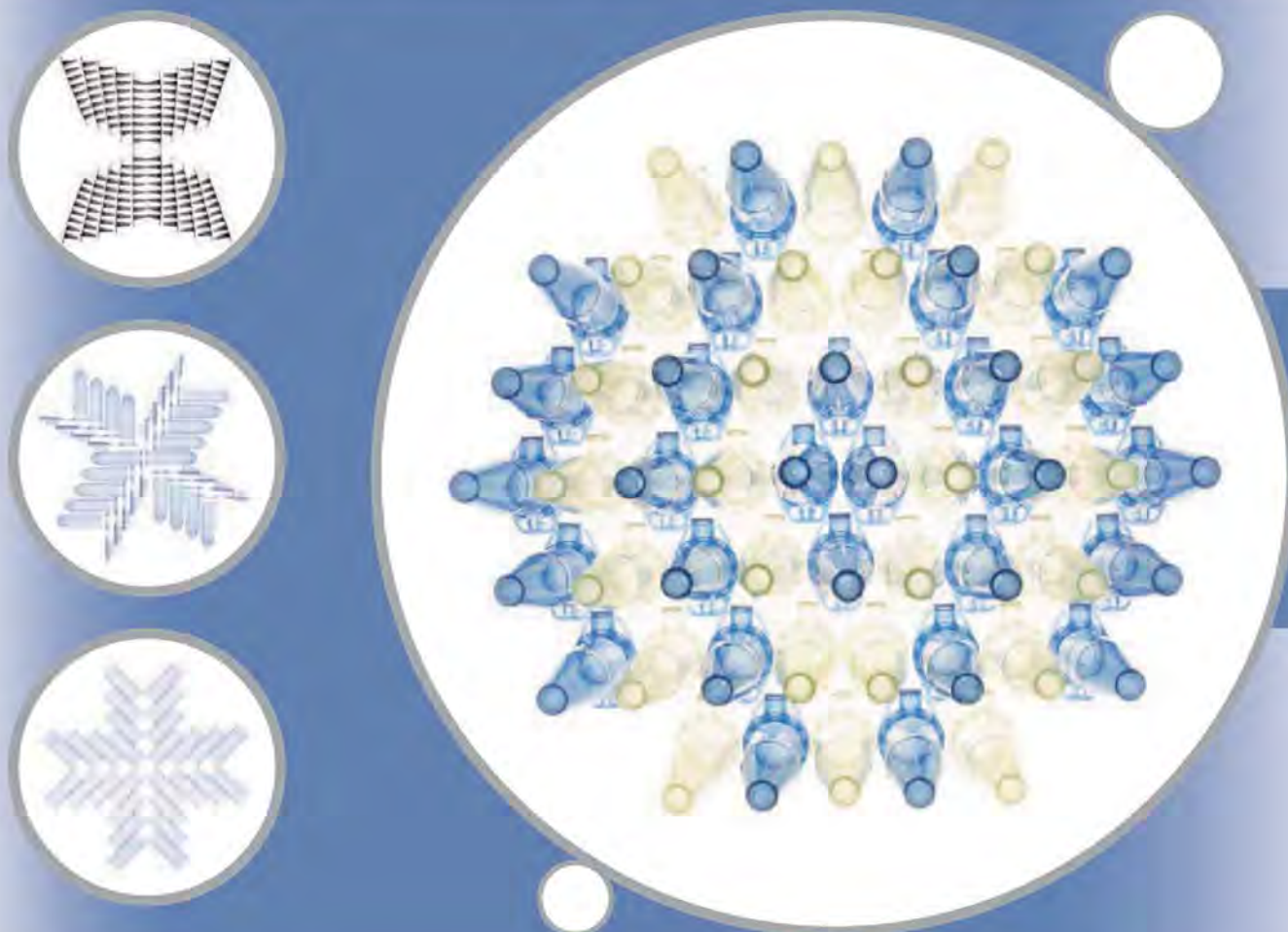
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Robert Zoellick is president of the World Bank Group in Washington, DC.

Development and Climate Change

NO COUNTRY IS IMMUNE TO CLIMATE CHANGE, BUT THE DEVELOPING WORLD WILL BEAR THE BRUNT of the effects, including some 75 to 80% of the costs of anticipated damages.* Millions in densely populated coastal areas and in island nations will lose their homes as the sea level rises, while poor people will face crop failures, reduced agricultural productivity, and increased hunger, malnutrition, and disease. Extreme events such as droughts, floods, and forest fires will become more frequent, making it even harder for developing countries to attain the United Nations' Millennium Development Goals of 2015. A "climate-smart" world is possible in our time. But to ensure a safe and sustainable future, all nations must act now, act together, and act differently.

The world must act now because actions today determine both the climate of tomorrow and the range of choices available to shape the future. The window for limiting temperature increases to a tolerable range is closing. All countries must act together, in a differentiated and equitable way, because tackling climate change involves diverse actions by many parties, with everyone being affected. Transforming the energy systems of the world requires ingenuity and cooperation on an unprecedented scale. Developed countries produced most of the greenhouse gas emissions of the past and currently have high per capita emissions. They must lead in both reducing emissions and financing mitigation and adaptation. Yet globally, most future emissions will be generated in the developing world. Those countries need adequate investments and technology so they can pursue lower-carbon paths without jeopardizing economic growth. We must face the reality that 1.6 billion people in the developing world still have no access to electricity and may not currently have low-carbon alternatives.

Countries must also act differently, particularly with respect to technology, education, and conservation. Planning for the future based on the climate of the past will erode development gains, deepen vulnerabilities, and increase inequities. Instead, we need innovation to invest in a technological revolution that can rapidly provide options for adaptation and mitigation, as well as new institutions and "market pull" policies to encourage entrepreneurship. Scaled-up, innovative financing that leverages funds from other investments, including in the private sector, is critical. We need more centers of excellence to build capacity across public and private sectors to enable innovative education programs, technologies, market solutions, and management practices. Tomorrow's scholars will have to integrate disciplines such as ecology, meteorology, public policy, business, finance, urban planning, agriculture, and public health if they are to solve complex and interrelated environmental and economic problems in concert with climate change. All countries must confront burgeoning demands on land and water, providing people with food, shelter, and energy and protecting biodiversity. Adaptation will require exploring genetic varieties of crops that can withstand new climate extremes, creating conservation corridors to facilitate species' migration, and providing incentives to preserve ecosystem services.

The World Bank Group is extending support in numerous innovative ways to effect change. These include Climate Investments Funds, a Clean Technology Fund, and direct Bank Group financing in support of expanded loans and grants for renewable energy and energy efficiency (<http://beta.worldbank.org/climate/>). For example, in Turkey, a company has built a waste management and recycling plant that generates not only biomass fuel but compost that is in turn used to grow vegetables for sale. Other initiatives include a solar thermal hybrid project in Egypt (in partnership with the Global Environment Facility, a group of nearly 200 countries, institutions, private-sector companies, and nongovernmental organizations), a sustainable environmental management project in Brazil, and a pilot BioCarbon Fund in Madagascar and several other African countries. But more is needed.

If the global community acts now, together, and differently to join scientific knowledge with cross-disciplinary collective solutions and innovative development strategies, we can indeed shape our climate future.

— Rosina M. Bierbaum and Robert B. Zoellick

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ASTRONOMY

Gazing Through the Dust

Galaxies undergoing vigorous bursts of star formation are often optically concealed by dust but appear luminous at longer wavelengths because the dust absorbs the light of the stars and re-emits it in the far infrared. If the galaxies are distant, they are detected at submillimeter wavelengths near Earth, because the far-infrared light gets stretched (or redshifted) by the cosmological expansion of the universe. In this way, hundreds of dusty, star-forming submillimeter galaxies have been detected over the past decade. Determining their precise distances from Earth, however, requires measuring a spectrum of their starlight, which is often too faint to detect.

As a proof of concept for circumventing this problem, Wei *et al.* used the new receiver at the IRAM 30-m radio telescope to look for CO emission lines—arising from the molecular gas that fuels star formation—from a submillimeter galaxy that was discovered in 1998 but for which it has not been possible to determine a distance. Their detection of two emission lines places the submillimeter galaxy SMM J 14009+0252 at redshift 2.93, or 11.4 billion light years away, and showcases a promising technique for determining the redshifts of submillimeter galaxies. — MJC

Astrophys. J. **705**, L45 (2009).

ENGINEERING

Wide Yet Sensitive

In a displacement force sensor, there is an inherent tradeoff between sensitivity (which requires large deflections to detect weak applied forces) and bandwidth (which requires small deflections to accommodate a wide range of applied forces). Similar tradeoffs apply to sensing in more than one direction while keeping the device compact. Wood *et al.* sought to optimize these tradeoffs in force sensor design. They fabricated a three-dimensional sensor

by folding a laser-patterned Invar sheet, reinforcing it with glass-filled epoxy, and soldering the tabs together. Numerical analysis revealed almost no coupling between the deformation responses along the orthogonal axes. The device proved more sensitive than expected from the modeling studies, because of a lower than expected stiffness, and the bandwidth was also affected by the weight of the adhesive used. The authors demonstrated the sensor's capabilities by simultaneously measuring the lift and drag forces from the flapping wing of a fly-sized robotic insect. — MSL

Smart Mater. Struct. **18**, 125002 (2009).

SIGNAL TRANSDUCTION

Single-Molecule Sensitivity

The external fertilization of sea urchin eggs relies on an extreme sensitivity of the sperm to chemoattractant molecules that guide the sperm toward the egg. In fact, *Arbacia punctulata* sperm can sense a single molecule of chemoattractant. Bönigk *et al.* show that such sensitivity is not limited to the receptor-type guanylyl cyclase that is located on the sperm cell membrane and detects the chemoattractant. This enzyme makes the second messenger cyclic guanosine monophosphate (cGMP), and the cGMP produced is detected with single-molecule sensitivity by K⁺-selective cyclic nucleotide-gated (CNGK) channels. Binding to only one of the four cGMP-binding sites on the tetrameric channel was sufficient to open the channel. The authors also deployed a caged form of cGMP, in which photolysis breaks the cage and releases cGMP with a concomitant fluorescence signal. This reagent allowed them to estimate that less than 50 molecules of cGMP were formed in response to a single molecule of chemoattractant. Only a small fraction of these are likely to diffuse and bind to a channel because of competing binding sites and degradation pathways in the cell. The authors propose that other high-affinity receptors for neurotransmitters and hormones may also display single-molecule sensitivity. — LBR

Sci. Signal. **2**, ra68 (2009).

ECOLOGY

Timing Is Everything

The mathematical description of coupled oscillations dates back to the 1665 analysis of pendulum clocks by Christiaan Huygens. Ecological models predict that populations of species in a community can exhibit similar oscillatory behaviors, giving rise to complicated patterns of synchrony and chaos in their dynamics. Benincà *et al.* provide an empirical confirmation of these predictions by studying a food web of phytoplankton prey and zooplankton predators that was maintained in the laboratory for 8 years. They observed that the chaotic dynamics of the populations were driven by the coupling of two predator-prey cycles, in which the larger phytoplankton species was eaten mainly by copepods and the smaller phytoplankton species was consumed by the relatively smaller zooplankton species—rotifers. Competition for resources between the phytoplankton species caused the predator-prey cycles to fluctuate out of phase, ultimately permitting the tenuous coexistence of the component species in the food web. Such principles may govern the longer-term, and hence less easily studied, dynamics of more intricate food webs involving longer-lived species. — AMS

Ecol. Lett. **12**, 1367 (2009).



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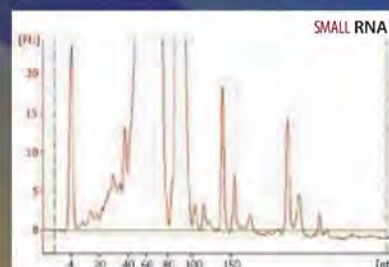
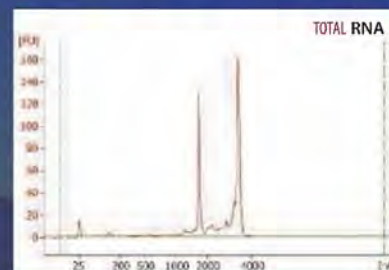
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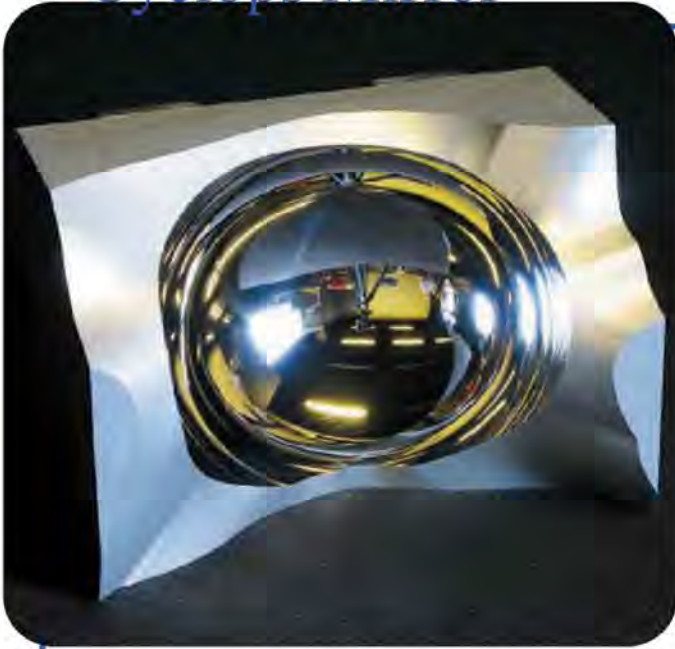
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Cyclops Mirror



Amsterdam-based artist and space engineer Bradley Pitts wanted to create a unique visual experience, but he may have also created a scientific instrument.

Pitts presented a strange, sphere-shaped mirror called the Ellipsoidal Introspective Optic (EIO) on 23 October during a meeting at the Royal Netherlands Academy of Arts and Sciences, which co-funded the €80,000 project. Pitts designed the mirror so that when you look into it, your right eye sees your left eye and vice versa—whichever direction you look. “You’re looking at the look-

ing itself,” Pitts says. Get close enough, and the images merge in your brain, turning your reflection into a Cyclops.

Utrecht University physicist Raymond van Ee plans to study how the brain handles the experience, which he describes as “very cozy and comfortable.” Van Ee wants to add tiny projectors to the EIO so each eye sees a different image and then study how the brain resolves the conflict. Pitts says he’s happy with the scientific interest—and a little worried that it will destroy the “enigma” he hoped to create.

Petri Dish Artists

What looks like a fractal design—or perhaps a kelp forest seen from a distance—is actually an art piece created by nonhuman painters. This growing colony of *Paenibacillus* bacteria and other “microbial art” made their debut on 22 October in a new online gallery, www.microbialart.com.

The gallery’s founder, evolutionary biologist T. Ryan Gregory of the University of Guelph in Canada, started making microbial art for a campus exhibit inspired by Charles Darwin, evolution, and biodiversity. He soon discovered others around the world using bacteria and fungi as an artistic medium, such as Eshel Ben-Jacob, a physicist at Tel Aviv University in Israel, who created this piece. Some draw patterns with solutions of luminescent bacteria. Others, like Ben-Jacob, grow colonies in nutrient-limited agar to encourage them to spread out in complex search patterns.



The gallery should “help visitors to gain an appreciation for the beauty of organisms that we normally never see,” says Gregory, “or indeed which we often fear.”

Scrap Metal Maps

To improve recycling rates, scientists track down waste metal by counting every ounce used in buildings or manufactured goods—a tedious task.

Environmental scientist Jason Rauch of Yale University has found a quicker way: combining global wealth data and satellite images of Earth’s nighttime lights. First, Rauch gathered existing data on the relationship between metal usage and gross domestic product. Next, he used GDP numbers and nighttime light levels, which correlate with GDP, to create the first global maps of potentially recyclable metals down to 10 square kilometers. Not surprisingly, the maps show that metals are concentrated in the developed world, Rauch reported online on 26 October in the *Proceedings of the National Academy of Sciences*. Although some of Rauch’s numbers are up to 300% off previous estimates, his method is faster and predicts metal usage in regions currently without data, says geologist W. David Menzie of the U.S. Geological Survey in Reston, Virginia.

THREE Q’S This week, Michael Green, a theoretical physicist at the University of Cambridge in the United Kingdom, became the Lucasian Professor of Mathematics, a position previously held by Isaac Newton, Paul Dirac, and, most recently, Stephen Hawking. An expert in superstring theory, Green answered a few questions from *Science*.

Q: Do you feel any pressure to live up to your predecessors’ reputations?

I suppose the ghosts of my predecessors are lurking in the background, but it would be debilitating to worry about emulating them!

Q: You were part of the so-called First String-Theory Revolution. What did you do?

In 1981, we showed that the nonsensical infinite quantities that have plagued all other attempts to unite quantum theory with gravity are absent in superstring theory. Furthermore, in 1984 we showed that other potential disasters, known as chiral anomalies, are absent in superstring theory, which therefore has the potential to consistently describe all the forces, including gravity, in an elegant manner.

Q: Will string theory ever be tested directly?

It depends what you mean by “directly.” One well-publicized hope is that predictions of string theory will be tested by particle physics experiments or by cosmological observations, although precise predictions are hard to come by. But string theory has evolved into a subject of broader relevance. One example is the recent description of high-energy collisions of heavy ions in terms of gravitational effects involving black holes. Such weird interrelationships are intriguing and point to further excitement to come.



Tobacco's subpoena:
Just saying 'No'

780

Flu vaccines for the
developing world

782

PHYSICS

Helium-3 Shortage Could Put Freeze On Low-Temperature Research

The weird effects of quantum mechanics often emerge at extremely low temperatures. So 3 years ago, Moty Heiblum, a physicist at the Weizmann Institute of Science in Rehovot, Israel, ordered a large "dilution refrigerator," which uses frigid liquid helium as a coolant and can chill tiny electronic devices to within a thousandth of a degree of absolute zero, or 1 millikelvin. But now the manufacturer, Leiden Cryogenics B.V. in the Netherlands, cannot deliver the completed fridge: It cannot get enough helium-3—100 liters of room-temperature gas, as it's sold on the market—to fill the rig.

Heiblum has fallen victim to a severe shortage of helium-3, the lighter isotope of the most inert element. Two weeks ago, he also lost about 15 liters of helium-3 from an existing fridge when an electronic valve failed. When Heiblum tried to buy more, a supplier in the United States turned him away and a European company wanted an unaffordable €1300 per liter, up from €100 just 2 years ago. "If this continues, then low-temperature physics will just disappear," Heiblum says.

No end to the shortage is in sight, however. In recent years the supply of helium-3 has dwindled, while the demand has skyrocketed—especially since 2002, when the U.S. Department of Homeland Security (DHS) and Department of Energy (DOE) began deploying thousands of helium-3-filled neutron detectors to help prevent the smuggling of plutonium and other radioactive materials into the country. In the short term, demand will likely top 65,000 liters per year, while supply will hover between 10,000 and 20,000 liters per year, according to a DOE study. The shortfall threatens several research fields, and DOE, the major supplier, is releasing the gas only to researchers with U.S. funding.

Helium-3 also fills neutron detectors at

large neutron-scattering facilities used to probe materials, such as the one at the new Japan Proton Accelerator Research Complex (J-PARC) in Tokai. The projected need for that application alone exceeds 100,000 liters over the next 6 years. J-PARC researchers need 16,000 liters of helium-3 to complete detectors for 15 of 23 beamlines, says J-PARC's Masatoshi Arai: "If we cannot get helium-3 and detectors, ... [then] we cannot perform sufficiently good experiments from the neutron facility at J-PARC, for

which we spent \$1.5 billion for construction."

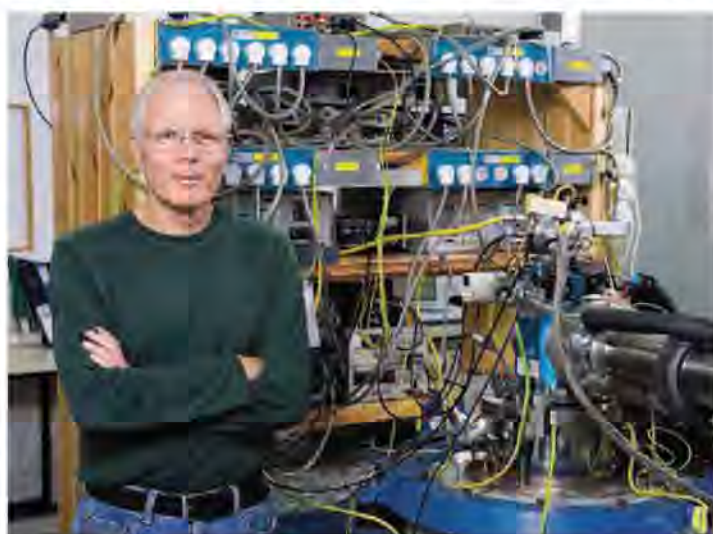
Low-temperature physicists say they need between 2500 and 4500 liters of helium-3 per year, primarily to fill new dilution refrigerators. Helium is the only substance that remains liquid at absolute zero, and only by pumping the vapor off a liquefied mixture of helium-3 and heavier helium-4 can physicists achieve steady temperatures below 0.8 kelvin, says William Halperin, a physicist at Northwestern University in Evanston, Illinois. "If we lose our helium-3 [supply], we're totally screwed," says Halperin, who notes that work on quantum computing and nanoscience often requires extremely low temperatures.

Helium-3 also serves a role in medical imaging. When inhaled by a patient, it allows researchers to image the lungs with an MRI.

The helium-3 supply likely won't return to its former levels. Rare in nature, the gas comes mainly from the radioactive decay of tritium generated in nuclear reactors. Pure tritium is an ingredient in hydrogen bombs, so for decades the United States and the Soviet Union kept large reserves of it and sold the helium-3 skimmed from it. But after the Cold War ended, the United States and Russia reduced their tritium reserves. Prior to 2009, DOE released 60,000 liters of helium-3 annually. In fiscal year 2009, it released 35,000 liters. Russia releases a few thousand liters annually, and Canada has accumulated tritium from civilian

reactors that, if purified, could provide a one-time boost of 80,000 liters of helium-3 and several thousand liters per year thereafter, DOE estimates.

To ease demand, researchers are looking for alternatives to helium-3, especially for neutron detectors for security. Within a sealed tube of gas, a helium-3 nucleus can absorb a passing neutron and then split into charged fragments that create a detectable electrical signal. Several alternative technologies exist, such as tubes filled with boron trifluoride gas or glass fibers that emit light when struck by a neutron. None of them is a "one-to-one replacement" for the helium-filled tubes in terms of sensitiv-



Global warming. Around the world, physicists like Moty Heiblum in Rehovot, Israel, say they cannot get the helium-3 they need to reach ultralow temperatures. Security programs in the United States have soaked up 85% of the supply.

HELIUM-3 USAGE IN THE PAST 5 YEARS

Low-temperature physics	1.3%
Medical imaging	1.7%
Oil & gas detectors	2.5%
Neutron-scattering, etc.	10%
Neutron detectors for security	84.5%





Science after the
Berlin Wall

788



A genome for
every vertebrate

794

ity, specificity, and ease of use, says Daniel Stephens, a nuclear engineer at Pacific Northwest National Laboratory in Richland, Washington. But as the projected demand for helium-3 for security alone outstrips the U.S. supply, DHS and DOE will likely begin to deploy other technologies soon, he says.

Meanwhile, an interagency working group convened by the White House has set three principles to guide the distribution of helium-3, says Steve Fetter, assistant director at-large at the White House's Office of Science and Technology Policy. DOE, which controls the U.S. supply, would give priority to uses for which helium-3 is irreplaceable, restrict its use to security applications in which it offers a decisive advantage, and give priority to projects in which

the United States already has a substantial investment, Fetter says.

That approach should ensure a supply for science. But it shuts out overseas researchers. Fetter says that "non-U.S. requests are not excluded from consideration." However, DOE brings helium-3 to market through Spectra Gases Inc. in Branchburg, New Jersey. In a 6 October e-mail message to a would-be buyer, Spectra Vice President Jack Faught said that DOE must approve sales of recently released helium-3 and that it "is reserved for research projects that are funded by certain specific agencies of the United States Government." Spectra did not reply to requests for comment.

That means scientists overseas cannot start new projects and equipment manufac-

turers may go out of business. "We only make dilution refrigerators," says Giorgio Frossati, co-founder of Leiden, one of three leading manufacturers of the devices. "If the helium-3 supply stops, it means that we have to close down." Under current conditions, Frossati says, the company, which employs 10 people and has annual receipts of €5 million, can hang on for a year.

Even if the current crunch can be ameliorated, the price of helium-3 will likely never return to the traditional \$100 to \$200 per liter. So certain types of research may become much more expensive. Meanwhile, reserves of helium-4 may be exhausted within decades, an even bigger problem that the National Academy of Sciences will weigh in on soon.

—ADRIAN CHO

U.S. SCIENCE POLICY

Peer Review Not Popular at Homeland Security

When it comes to funding basic research at the Department of Homeland Security (DHS), program officers seem to rely mostly on their own opinion. An analysis of the agency's \$1 billion science and technology directorate has found that only about 40% of the \$175 million spent on basic science was awarded through a competitive process. And only a portion of that slice is subject to outside peer review.

"That's not good for DHS and not good for science," says Cindy Williams, a political scientist at the Massachusetts Institute of Technology in Cambridge who chaired a study by the National Academy of Public Administration (NAPA) released earlier this year. Williams testified last week before the technology panel of the House of Representatives science committee at a hearing that looked at how DHS could do a better job of using science and technology. She recommended that the science directorate follow the example of the National Science Foundation and other agencies that award funds "on a competitive basis based on scientific peer review except in cases when that is clearly not feasible."

Asked at the hearing if DHS planned to follow that advice, Bradley Buswell, acting under secretary of the science directorate, was non-committal. "I agree that competition is good and that peer review is one means of assuring

that we are selecting high-quality projects," said Buswell. He noted that some DHS-funded research projects, including proposals funded through the agency's university-based Centers of Excellence program, follow this model. But he said that DHS's practice of reviewing research proposals internally was no less rigorous.

The NAPA study found that 31% of DHS's basic research funding last year went to universities. Even so, says Williams, "there is no peer review for individual projects funded by the centers" with money from their DHS award. Almost none of the remaining funds, divided among industry, federal labs, and other entities, was awarded after external peer review, Williams says.

Williams says directorate officials told her there were "only a few performers" who could meet the agency's needs. They also said few universities had the secure facilities needed to carry out classified research projects, although Williams notes that the bulk of the basic research funded by DHS is unclassified.

DHS's avoidance of outside advice is unusual even among security-conscious agencies. The Department of Defense uses peer review to disburse much of its \$1.5 billion



Peerless. A study led by Cindy Williams found that peer review is used infrequently by DHS to fund basic research.

basic research budget. And in recent years, U.S. intelligence agencies have tried to tap the research community by soliciting proposals and setting up external peer-review panels.

"We are a strong supporter of peer review," says Barry Toiv of the Association of American Universities. "It is probably a good idea for agencies to receive outside advice as they allocate their research funding."

—YUDHIJIT BHATTACHARJEE

STEM CELLS

CIRM Awards Seek to Move Cell Therapies to the Clinic

Five years after it launched, the California Institute for Regenerative Medicine (CIRM) last week awarded its first disease-oriented grants—\$230 million to 14 teams—intended to speed stem cell therapies to patients. In seeming contrast to its original mission, only about one-third of the 4-year

grants involve research using embryonic stem (ES) cells. The state created CIRM largely to fund ES cell research, which was lagging because of Bush-era restrictions on the controversial technology.

CIRM officials were quick to say that they never intended to fund only work on

In the mix. Only a few of CIRM's disease teams will use human embryonic stem cells like the colony here (light blue).

embryonic cells. Although “it is a priority to fill that gap,” said CIRM board chair Robert Klein at a press conference, “our commitment to the voters ... is that we would pursue the very best cell type for each disease.” CIRM President Alan Trounson proclaimed the mix of embryonic, adult, and cancer stem cell types funded “about the right proportion.”

To date, CIRM has funded basic research, infrastructure, and training; these awards are the first to support the preclinical work needed to bring a lab discovery to the point at which it's ready to be tested in patients. The goal of each “disease team” is to file a new drug application with the U.S. Food and Drug Administration within 4 years—much faster than the usual decade or more. CIRM has been shifting focus partly in response to the Obama Administration order earlier this year that lifted restrictions on using federal funds to study human ES cells (*Science*, 27 March, p. 1660).

PRIVACY

Court Orders Stanford Expert to Surrender Manuscript

A Stanford University professor is fighting to keep his unpublished book manuscript out of the hands of tobacco company R.J. Reynolds (RJR), which subpoenaed it after he testified as an expert witness for smokers who are suing the company. Last month, Stanford asked the court to reject the company's demand, saying that it could have a “chilling effect” on researchers serving as expert witnesses.

Scholars are often shocked and upset that their unpublished work is subject to subpoena, says Joe Cecil, senior researcher at the Federal Judicial Center in Washington, D.C., a government educational and research agency. But, especially if researchers offer themselves as expert witnesses, courts have wide-ranging powers to collect evidence. He adds, “Even though these kinds of cases have been around for decades, the scientific community doesn't really have an organized response.”

Stanford medical historian Robert Proctor plans to serve as an expert witness—either in person or via taped testimony—for smokers in many of the 8000 or so cases they are bringing against tobacco companies in

Florida. (For technical reasons, a state judge threw out a class-action lawsuit by Florida smokers a few years ago, so plaintiffs resorted to individual suits.)

Proctor has become a popular witness because of his expertise on the history of tobacco in the United States and because of his provocative criticisms. He calls tobacco companies the biggest environmental polluters on the planet and claims that “the cigarette is the most carefully designed artifact in the history of civilization, with the possible exception of nuclear bomb cores.” Since December, tobacco companies have lost seven of the nine cases tried in Florida, some of which included Proctor as a witness, for an average of a few million dollars each time. All the decisions are pending appeal.

Proctor has also been writing an 800-page book titled *The Golden Holocaust: How cigarette makers engineered a global health catastrophe*. The manuscript, which he hopes to finish early next year, so far consists largely of Proctor's private jottings and notes, he says. He plans a wide-ranging account of the tactics tobacco companies

used to sell cigarettes, including his views on how tobacco companies used science as a rhetorical tool. Much of Proctor's research comes from scientific studies and other documents in the tobacco companies' own files, which were secured by plaintiffs in anti-tobacco litigation and posted online.

“Companies linked themselves with an open-research ideology: ‘Let's keep our minds open to the possibility that smoking doesn't cause cancer,’” Proctor says. “It was a brilliant system that captured a lot of academics.” Companies sponsored millions of dollars of research as well into alternative explanations for cancer, he says, including mites, viruses, baldness, and month of birth.

RJR asked William Parsons, a circuit judge in Florida's Volusia County, to issue a subpoena for *The Golden Holocaust*. “When you become a paid expert witness, your notes, your correspondence with other people become available,” says Theodore Grossman, a partner at the law firm representing RJR, Jones Day in Cleveland, Ohio. “They're needed in order to conduct an effective cross-examination.” Adds Cecil, “Frankly, if [Proctor is] willing to take on the

CREDIT: CIRM

The 14 projects include four using human ES cells to treat stroke, type 1 diabetes, macular degeneration, and Lou Gehrig's disease; a fifth project is using an alternative to embryonic cells, induced pluripotent stem cells, for a skin disease. Other teams are using adult stem cells—sometimes combined with gene therapy—to treat diseases such as brain cancer, heart disease, and HIV/AIDS. Three grants will target cancer stem cells. The one award to a biotech company, \$20 million to Novocell Inc. in San Diego, for the diabetes team, is actually a loan.

Donald Kohn of the University of California, Los Angeles (UCLA), who heads a \$9 million project using gene therapy to treat sickle cell disease, says the awards are important because the money will support the entire range of expertise needed to move a therapy into clinical trials, including regulatory staff. The three CIRM grants to UCLA “will make [the research] go a lot quicker,” said Kohn in the press conference.

Arnold Kriegstein of the University of California, San Francisco, who has wor-

ried that CIRM is pushing too hard to move stem cells into the clinic, is encouraged by the focus on both embryonic and adult stem cells, which are further along. But he still worries about CIRM's “shift towards higher risk preclinical studies at the expense of the huge amount of basic research that needs to be done.”

At the same time, the National Institutes of Health (NIH) is moving closer to funding its first grants under expanded federal guidelines on ES cells issued on 7 July. The holdup is that NIH hasn't decided which lines are eligible for funding.

NIH has received submissions from seven institutions seeking approval for 91 cell lines and is “moving expeditiously” to see if the lines meet the spirit of new ethical rules, an NIH official says. A working group of the NIH director's advisory committee will submit recommendations to the full committee in December, which will then send its advice to NIH Director Francis Collins. Those lines derived from embryos donated since 7 July in accordance with the new guidelines may be approved sooner, the NIH official says.

—JOCELYN KAISER

role of an expert who's compensated, ... he's consented to a much more searching inquiry.” But Proctor has been fighting the subpoena since August.

A request for unpublished scientific research is unusual but not unprecedented. In recent years, scientists who study lead paint, drugs, and toxic chemicals have received subpoenas for raw data in tort cases. Some capitulated, and a few left academe after bad experiences. At times, companies have even subpoenaed peer reviewers of scientific manuscripts. Proctor's case differs from most in that he conducted no independent experiments, just analyzed tobacco companies' own work.

In an amicus curiae brief filed on 8 October with the Florida court to support Proctor, Stanford's dean of research, Ann Arvin, urged that the subpoena be dropped: “Stanford respectfully urges the Court to consider the broad societal interest that academic researchers not be forced to pre-publish their uncompleted research,” which could harm their professional standing.

Grossman argues that courts have effective rules to prevent the dissemination of unpublished work. “If Proctor had approached us to get a confidentiality seal, I'm sure we could have worked something out. He didn't. ... Proctor apparently does



Standoff. Robert Proctor has refused a tobacco company's demand for his unpublished research.

not trust the rules of the courtroom.”

Proctor scoffs at RJR's claim that tobacco companies need more evidence about his views. He remains adamant that he will not surrender the bulk of his unedited, unfiltered manuscript, which he says would violate his privacy and First Amendment rights. The issue will likely be resolved over the next few weeks.

—SAM KEAN

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From Science's Online Daily News Site

A Little Fellatio Goes a Long Way

Oral sex is surprisingly rare in the animal kingdom. Humans do it, of course, as do bonobos, our close relatives.

But now

researchers have observed the practice for the first time in a nonprimate. During intercourse, female short-nosed fruit bats lick the genitals of their partner, a possible ploy to increase copulation time. The discovery suggests that there may be a biological advantage to fellatio. <http://bit.ly/batfellatio>



A Body Count for Two Man-Eating Lions

For 9 months in 1898, two lions terrorized the southern Kenyan region of Tsavo, killing as many as 135 people by one account. Although the almost mythic tale has spawned three movies, people still debate the final death toll. Now, hair and bone samples from the famed creatures have shed light on how many people they devoured and why they did it. <http://bit.ly/maneaters>

Did Ancient Earth Go Nuclear?

A surge of oxygen littered early Earth with millions of tiny nuclear reactors, blasting ancient life with radiation. That's the scenario a team of researchers has proposed to account for the disappearance of a radioactive mineral from the geological record. If true, this primordial nuclear age could have played a role in the evolution of early life forms. <http://bit.ly/nuclearearth>

A Concise and Precise Definition of P-Value

Victor De Gruttola, the chair of biostatistics at the Harvard School of Public Health, is passionate about his p-values. But journalists, he says, often get the concept wrong. To heal wounds and improve communications between biostatisticians and the confused masses that rely on them, De Gruttola agreed to discuss the details of what a p-value means and does not mean with ScienceNOW. <http://bit.ly/pvalue>

Read the full postings, comments, and more on scienown.sciencemag.org.



SWINE FLU PANDEMIC

Developing Countries to Get Some H1N1 Vaccine—But When?

When Margaret Chan addressed the United Nations General Assembly on 4 May, just weeks after swine flu had surfaced, she ended on a solemn note. “An influenza pandemic is a global event that calls for global solidarity,” the director-general of the World Health Organization (WHO) said. “It is my job to do whatever is possible to ensure that developing countries are not left without protection.”

Six months later, many global health experts conclude that, despite all her hard work, Chan has precious little to show for it. WHO has said that about a month from now, it will begin supplying developing countries with H1N1 vaccine donated by manufacturers and rich countries. But it has secured only about 200 million doses for 95 countries that together are home to a third of the world’s population. Compare that with the 250 million doses that the United States has purchased, Germany’s 50 million, or France’s 94 million, and “global solidarity” aren’t the first words that come to mind. Moreover, much of that vaccine won’t arrive for several months, and the pandemic marches on.

“It’s probably going to be too little, too late,” says David Fidler, an international law professor at Indiana University, Bloomington, and an expert on global health security. Tido von Schoen-Angerer, who directs the Campaign for Access to Essential Medicines for Doctors Without Borders (MSF) in Geneva, calls WHO’s operation “largely symbolic.” Years of debate and negotiations have failed to produce a system to ensure equal access to the lifesaving vaccines, he says, leaving WHO to distribute mere table crumbs. The only good news, he

adds, is that the pandemic is relatively mild.

A month before its scheduled start, many details of WHO’s operation are still lacking. The agency won’t make public the list of eligible countries, says Marie-Paule Kieny, director of the WHO Initiative for Vaccine Research, because some of the 95 may opt out of the plan. In fact, only about 40 countries so far have formally indicated to WHO that they want the vaccine, she says. Some have qualms about the fact that they have to assume liability for adverse events—a condition that the vaccine manufacturers have also imposed on rich countries. “This is creating a lot of discussion,” says Kieny. “Governments want to know what it means and whether the vaccine is safe.”

There are uncertainties on the supply side as well. Two big vaccine manufacturers, GlaxoSmithKline (GSK) and Sanofi Pasteur, have pledged 50 million and 100 million doses, respectively, whereas two smaller players, MedImmune and CSL, will each pitch in 3 million doses. The size of the donation from the 10 individual countries that have promised to support WHO remains unclear, however. Some, such as the United States and Australia, have said they will donate 10% of their own supply, but others have not made a firm commitment. Some may donate money instead, Kieny says: “We have to see who is going to do what.”

It’s also unclear when the pledged vaccine will materialize. The timing is essential, given the fast-moving nature of the pandemic. Kieny says that GSK shipments will start in late November and last through April or May; Sanofi Pasteur has promised to start before the end of the year, she says. The U.S. government

Priorities. U.S. lacks enough vaccine to meet demand, as crowds in Maryland found out on 21 October.

has made conflicting statements about when it will start providing its 25 million doses, but it’s facing shortages itself and is under increasing political pressure to take care of its own population first. “It’s going to be a very tricky decision-making process for any government,” says former Merck executive Adel Mahmoud, now a researcher at Princeton University.

WHO expects that between late November and February, when most eligible countries in the Northern Hemisphere will be hit hardest, at least 37 million doses will be available for them, enough to vaccinate 2% of the population. That means the vaccine will at least have an impact among health care workers, the group a WHO panel has said should be first in line. But “there is still hope” that other risk groups can be protected in time as well, Kieny adds. In African countries that see few international travelers, the pandemic peak may come later, for instance, and in Southern Hemisphere countries, the vaccine may be useful during the second wave, expected to hit there in the first half of 2010. In any case, she says, “it’s better than nothing.”

Because the swine flu pandemic has been mild so far, the lack of access to the vaccine has not yet ignited the international political explosion some had feared. Developing countries have not stood up to demand vaccine, at least not in public, and not a single country has threatened to withhold H1N1 virus samples from WHO’s global lab network until vaccine supply is guaranteed, as Indonesia did in the past with samples of the deadly H5N1 avian influenza virus. MSF and other nongovernmental organizations have remained mostly silent on the issue because it pales in comparison to the importance of access to AIDS and malaria drugs. The donations, although modest, probably helped to “let off some of the political steam,” says Fidler.

But the world may not be so lucky next time—and few doubt that rich countries would have shown far less largess if the pandemic had been very severe. That’s why it’s important to increase the global production capacity for influenza vaccine and get rid of the antiquated production methods based on hens’ eggs, Mahmoud says. One thing that might change the dynamic, says Fidler, is that new countries are entering the vaccine market. “China is now a big manufacturer,” he says. “India has scientific expertise. Brazil is becoming an important player. You may see some movement between the developing countries. We no longer have to rely on these difficult north-south relationships.”

—MARTIN ENSERINK

CREDIT: AFP/GETTY IMAGES/NEWS.COM

ECONOMIC RECOVERY

When Counting Jobs Isn't Enough

Keeping track of the number of jobs created and saved by the \$787 billion stimulus package is a staggering challenge for the Obama Administration. It's also extra work for grant recipients, who must report how they're spending the money and its impact on scientific employment.

A federal pilot project launched this summer aims to increase the accuracy of the count and reduce the burden on U.S. research universities. If it succeeds, it could also lead to a better way of tracking how federal investments in science affect the country's long-term economic health.

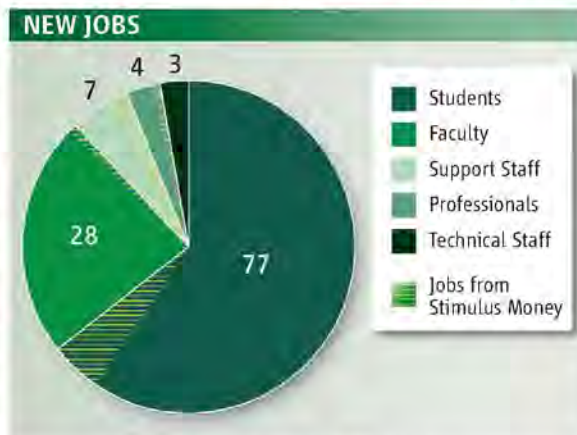
Last week, Administration officials reported that the initial \$159 billion chunk of spending from the American Recovery and Reinvestment Act (ARRA) has created or saved 640,329 jobs. A small number of them are at universities and other institutions receiving part of the \$20 billion slice of the recovery package flagged for research and science infrastructure projects. After filling out the first round of reports, due 30 September, university officials are exhausted—and frustrated.

"I'm a vice president, and I personally completed every one of our 100-plus forms," says Susan Sedwick, who oversees sponsored research at the University of Texas, Austin. "The guidelines are geared more to the construction industry and those receiving contracts than to the university environment," she explains, noting differences in the definition of a job and how to count full-time equivalent positions. "My hat's off to OMB [the White House's Office of Management and Budget] for putting in a new system that did not crash. But we all need better guidance on what to do."

Clearing up those ambiguities is one of the goals of the Science and Technology for America's Recovery pilot project being run out of the White House Office of Science and Technology Policy (OSTP). Working with data from seven universities on all federal awards, the project has shown that it's possible to extract the information needed for ARRA from the numerous accounting systems already used by universities, says Julia Lane, a labor economist at the National Science Foundation who is coordinating the effort. The result is a more rigorous and accurate tally.

"You don't ask the PIs [principal investigators], you follow the money," Lane explains. "The finance department already has data on every award, and every pay period we know who's charged their time to that code. If they show up in the previous pay period, it's a job saved. If not, it's a job created. There's no argument—and it's completely auditable."

The next step is to scale up the system throughout the country to track ARRA spending, she says. OSTP Director and presidential science adviser John Holdren wants to go even further, to obtain a broader picture of what science can do for the country. In particular, Holdren would like to see the tracking system expanded to all awards, not just for ARRA-funded work,



A summer surge. New student jobs led the hiring at one university in the sample, which added 119 jobs in July from all science awards. Of those, 10 were created by recovery funds.

and combined with outcomes such as publications, patents, and spinoff companies. The result, Holdren says, would be to "increase the evidence base ... on the cumulative impact of science investments on the nation's R&D work force and the role of science in [global] competitiveness."

Such a system would require the cooperation of researchers whose efforts are being tracked. That's already happening, says Michael Laskofski of George Mason University in Fairfax, Virginia, which is part of the pilot project. "One of the reasons I was interested in participating is that we're an emerging research university," says Laskofski, noting that the school so far has received 21 ARRA awards. "And our faculty understand the importance to the nation of collecting this type of additional information."

—JEFFREY MERVIS

ScienceInsider



From the Science Policy Blog

The National Institutes of Health (NIH) has decided to fund 840 of the more than 20,000 applications it received for its vaunted **Challenge Grants** program. That 4% success rate may be abysmal compared with the one-in-five rate for regular NIH grants, but it's more than twice as high as NIH's original projections. <http://bit.ly/2uTQ5X>

Critics of the **Large Hadron Collider** at CERN on the Switzerland-France border will petition the United Nations human rights committee to stop the facility from restarting because of risks that the collisions could create dangerous mini-black holes. Independent physicists say such fears are groundless. <http://bit.ly/4im5gD>

A new clinical trial will explore whether a drug could reverse mental retardation. The federally funded trial by a Massachusetts company will enroll healthy volunteers to assess the safety of a proposed treatment for **Fragile X syndrome**, the most common cause of mental disability. <http://bit.ly/35jq17>

The new budget for the Interior Department includes a request by Congress that the department obtain an independent **assessment of ecological risks** of oil and gas exploration in the Arctic. <http://bit.ly/1nCAya>

The top **drug policy** adviser to the British government was fired after he spoke out against current policy on illegal substances. A number of members of the Advisory Council on the Misuse of Drugs have quit in protest. <http://bit.ly/3UelK1>

Scientists who study viruses in the natural environment are facing what one called a "nightmare" of **disrupted research projects**. Filters made by a company named Whatman were crucial to their work, but GE Healthcare decided to discontinue production after purchasing the company last year. <http://bit.ly/2diCbh>

For more science policy news, visit blogs.sciencemag.org/scienceinsider.

On the Origin of Religion



To Charles Darwin, the origin of religious belief was no mystery. "As soon as the important faculties of the imagination, wonder, and curiosity, together with some power of reasoning, had become partially developed, man would naturally crave to understand what was passing around him, and would have vaguely speculated on his own existence," he wrote in *The Descent of Man*.

But our propensity to believe in unseen deities has long puzzled Darwin's scientific descendants. Every human society has had its gods, whether worshipped from Gothic cathedrals or Mayan pyramids. In all cultures, humans pour resources into elaborate religious buildings and rituals, with no obvious boost to survival and reproduction. So how and when did religion arise?

No consensus yet exists among scientists, but potential answers are emerging from both the archaeological record and studies of the mind itself. Some researchers, exploring religion's effects in society, suggest that it may boost fitness by promoting cooperative behavior. And in the past 15 years, a growing number of researchers have followed Darwin's lead and explored the hypothesis that religion springs naturally from the normal workings of the human mind. This new field, the cognitive science of religion, draws on psychology, anthropology, and neuroscience to understand the mental building blocks of religious thought. "There are functional properties of our cognitive systems

that lean toward a belief in supernatural agents, to something like a god," says experimental psychologist Justin Barrett of the University of Oxford in the United Kingdom.

Barrett and others see the roots of religion in our sophisticated social cognition. Humans, they say, have a tendency to see signs of "agents"—minds like our own—at work in the world. "We have a tremendous capacity to imbue even inanimate things with beliefs, desires, emotions, and consciousness, ... and this is at the core of many religious beliefs," says Yale University psychologist Paul Bloom.

Meanwhile, archaeologists seeking signs of ancient religion focus on its inextricable link to another cognitive ability: symbolic behavior. They, too, stress religion's social component. "Religion is a particular form of a larger, social symbolic behavior," says archaeologist Colin Renfrew of the University of Cambridge in the United Kingdom. So archaeologists explore early religion by excavating sites that reveal the beginnings of symbolic behavior and of complex society.

Yet these fields are developing chiefly in parallel, and there remains a yawning gap between the material evidence of the archaeological record and the theoretical models of psychologists. Archaeological objects fall short of revealing our ancestors' minds, says Bloom, while on the psychological side, "we need more evidence."

Birth of the gods

When did religious beliefs begin? A likely place to find out is the archaeological record, but inferring "religion" from ancient objects and practices can be a tall order. Many researchers take the use of symbols as a clue to budding spirituality. As far back as 100,000 years ago, people at the South African site of Blombos Cave incised pieces of ochre with geometric designs, creating the first widely recognized signs of symbolic behavior (*Science*, 30 January, p. 569). Although it's difficult to equate enigmatic lines on a chunk of ochre with a belief system, researchers agree

that such use of symbols is a prerequisite for religion, and some argue that religious beliefs must have existed by this time.

The first deliberate burials are found at roughly the same time, at a site called Qafzeh in Israel, dated to about 95,000 years ago. Researchers have dug up more than 30 individuals, including a 9-year-old child with its legs bent and a deer antler in its arms. And starting about 65,000 years ago or even earlier, Neandertals also sometimes buried their dead. Henry de Lumley of the Institut de Paléontologie Humaine in Paris has referred to these ancient burials as "the birth of metaphysical anguish."

But others aren't sure what metaphysical message burial conveys. "There can be lots of reasons to bury things; just look at kids in a sandbox," says Barrett. Burial by itself, says archaeologist Nicholas Conard of the University of Tübingen in Germany, may best be considered a sign of "protobelief."

If they had to name one time and place when the gods were born, Conard and some others might point to 30,000 to 35,000 years ago in Europe. That's when symbolic expression flowered in what's called the Upper Paleolithic explosion (*Science*, 6 February, p. 709). At this time, Ice Age hunter-gatherers painted strikingly realistic animals—and a few half-animal, half-human figures—on the walls of France's Grotte Chauvet and other caves. They also left small but spectacular figurines in caves in Germany, including a dramatic carved ivory "Venus" reported in May and three "lion-men"—each a carved male body with the head of a lion.

The "Venus of Hohle Fels" illustrates the difficulties of interpreting such ancient objects: Conard, who discovered it, considers

the 6-centimeter figure of a headless woman with huge breasts and carefully carved genitalia to be a religious fertility object, while archaeologist Paul Mellars of the University of Cambridge has called it "paleo-porn."

Yet many observers agree that the lionmen, with their combination of human and animal qualities—something seen in many early religions—are strong candidates for a supernatural being or spirit guide. Some go so far as to suggest that the small statues were part of shamanistic rituals, though Conard says we cannot know for sure. "Even if it wasn't shamanism," he says, "I'd bet the bank it was something I'd consider religious beliefs."

THE YEAR OF DARWIN



This essay is the 11th in a monthly series. For more on evolutionary origins online, see the Origins blog at blogs.sciencemag.org/origins. For more on the Origin of Religion, listen to a podcast by author Elizabeth Culotta at www.sciencemag.org/multimedia/podcast.



The world over. All cultures have religious beliefs, though they express them in diverse ways.

Twenty thousand years later, humans reached another religious milestone, building what is often considered the world's first temple at the 11,000-year-old site of Göbekli Tepe in Turkey (*Science*, 18 January 2008, p. 278). There, rows of standing stones up to 6 meters tall march down a high hillside in circles; each massive stone is carved with images of wild animals. "There is the erection of monumental and megalithic architecture for the first time," says excavator Klaus Schmidt of the German Archaeological Institute in Berlin.

After this time, more organized sites with apparently religious aspects appear elsewhere. For example, at one of the first settled towns, Çatalhöyük in southern Turkey, excavator Ian Hodder of Stanford University and his crew are finding what they consider copious evidence of spiritual life: feasts with wild bulls, burials of ancestors beneath houses, and sometimes the removal and reinterment of skulls. And yet Hodder notes that separating "religion" from other activities seems arbitrary, as it is not clear that the people of Çatalhöyük themselves separated the religious sphere from the rest of life.

Renfrew cautions that it might not be possible to know for sure that a culture worshipped gods until we can read their names—that is, until the literate societies of ancient Mesopotamia and Egypt, some 5000 years ago. Those early empires had both secular and religious hierarchies, with priestly elites and sometimes a god-king who ruled both the temporal and spiritual realms. In this view, full-fledged "religion" develops hand in hand with organized social hierarchies. It may be that "you don't necessarily have belief in deities until you have persons of enormously high status, who themselves are close to divine," like a pharaoh, says Renfrew.

Born believers?

While archaeologists trace the outward expressions of religious and symbolic behavior, another group of researchers is trying to trace more subtle building blocks of religious belief, seeking religion's roots in our minds.

"You begin to see that a god is a likely thing for a human mind to construct."

—Deborah Kelemen,
Boston University

According to the emerging cognitive model of religion, we are so keenly attuned to the designs and desires of other people that we are hypersensitive to signs of "agents"; thinking minds like our own. In what anthropologist Pascal Boyer of Washington University in St. Louis in Missouri has described as a "hypertrophy of social cognition," we tend to attribute random events or natural phe-

nomena to the agency of another being.

When it comes to natural phenomena, "we may be intuitive theists," says cognitive psychologist Deborah Kelemen of Boston University (BU). She has shown in a series of papers that young children prefer "teleological," or purpose-driven, explanations rather than mechanical ones for natural phenomena.

For example, in several studies British and American children in first, second, and fourth grades were asked whether rocks are pointy because they are composed of

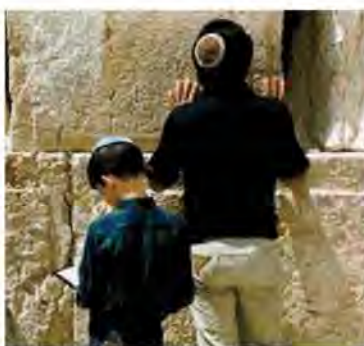
small bits of material or in order to keep animals from sitting on them. The children preferred the teleological explanation. "They give an animistic quality to the rock; it's protecting itself," Kelemen explains. Further studies have confirmed this tendency. Even Kelemen's own son—who "gets mechanistic explanations of everything"—is not immune: At age 3, after hearing how flowers grow from seeds, his question was, "Who makes the seeds?"

The point of studying children is that they may better reflect innate rather than cultural biases, says Kelemen. But recent work suggests that it's not just children: Kelemen and Krista Casler of Franklin & Marshall College in Lancaster, Pennsylvania, found the same tendency to ascribe purpose to phenomena like rocks, sand, and lakes in uneducated Romany adults. They also tested BU undergraduates who had taken an average of three college science classes. When the undergrads had to respond under time pressure, they were likely to agree with nonscientific statements such as "The sun radiates heat because warmth nurtures life."

"It's hard work to overcome these teleological explanations," says Kelemen, who adds that the data also suggest an uphill battle for scientific literacy. "When you speed people up, their hard work goes by the wayside." She's now investigating how professional scientists perform on her tests. Such purpose-driven beliefs are a step on the way to religion, she says. "Things exist



Signs of the spirit? Small, 30,000-year-old figurines from Germany suggest religious belief.



for purposes, things are intentionally caused, things are intentionally caused for a purpose by some agent. ... You begin to see that a god is a likely thing for a human mind to construct."

Other researchers find the work intriguing. "If her data are right, we all from childhood have a bias to see the natural world as purposefully designed," says Barrett. "It's a small step to suppose that the design has a designer."

This predisposition to "creationist" explanations has resonance with another tendency in the human mind, says Barrett—something he calls the "hypersensitive agency detection device": looking for a thinking "being" even in nonliving things. In classic experiments in the 1940s, psychologists found that people watching animations of circles, triangles, and squares darting about could identify various shapes as characters and infer a narrative. Anthropologist Stewart Guthrie noted in 1993 that this tendency could help explain religion, because it implies we attribute "agency" to all kinds of inanimate objects and ambiguous signals. As Barrett describes it: "When I hear a bump in the night, I think 'Who's there?' not 'What's

there?' ... Given ambiguous stimuli, we often posit an agency at play."

Guthrie suggested that natural selection primed this system for false positives, because if the bump in the night is really a burglar—or a lion—you could be in danger, while if it's just the wind, no harm done.

Of course, this is still a long way from believing in gods or spirits. But a hair-trigger agency detector could work with another sophisticated element of the human mind to make us prone to believe in gods, cognitive researchers say. They refer to what's called theory of mind, or the understanding that another being has a mind with intentions, desires, and beliefs of its own. Studies have shown that this ability develops over time in children and is usually present by age 5; functional magnetic resonance imaging (fMRI) studies have localized the parts of the brain involved.

If you suspect that an agent was responsible for some mysterious event, it's a short step

to thinking that the agent has a mind like your own. "Higher order theory of mind enables you to represent mental states of beings not immediately or visibly present,

and who could have a very different perspective than your own," says Barrett. "That's what you need to have a rich representation of what it might be like to be a god." (It's also what is needed to have a functional religion, because people need to know that others share their beliefs.) As Darwin put it, humans developing religion "would naturally attribute to spirits the same passions, the same love of vengeance, or simplest

form of justice, and the same affections which they themselves feel."

Some fMRI studies lend support to this idea. In the 24 March issue of the *Proceedings of the National Academy of Sciences*, a team led by Jordan Grafman of the National Institute of Neurological Disorders and Stroke in Bethesda, Maryland, asked 40 people to evaluate statements about God's emotions and relationships to humans, such as, "God is removed from the world" and "God is forgiving," while they were in an fMRI scanner. The researchers found that the areas that lit up (indicating oxygen uptake and so presumably brain activity), such as the inferior frontal gyrus on both sides of the brain, are also involved in theory of mind. This and other results argue against any special "god region" of the brain as some have suggested, says Grafman. Rather, he says, "religious belief co-opts widely distributed brain sectors, including many concerned with so-called theory of mind."

Other researchers are extending this cognitive model, finding additional thought processes that they say make religious belief natural. For example, Bloom and Jesse



Who made it? Studies suggest that children tend toward creationist explanations of natural phenomena.



Raising the temple. The standing stones at Göbekli Tepe are considered by many to be the oldest humanmade holy place.

CREDITS: (TOP FROM LEFT TO RIGHT) WIKIPEDIA (2); JUPITERIMAGES; WIKIPEDIA (2); (FROM TOP TO BOTTOM) JUPITERIMAGES; BERTHOLD STEINHILBER/REDFX



Bering of Queens University Belfast argue that children are predisposed to think that the mind persists even after the death of the body—something that approaches the idea of an afterlife. Bering showed children ages 4 through 12 years old a puppet show in which a crocodile ate a mouse. Then he asked the children questions about the mouse. Did it feel hunger? Was it still mad at its brother? The children agreed that the mouse's body no longer functioned; it didn't need to eat, for example. But they thought it would still feel hunger; its psychological states persisted. Preschoolers showed this tendency more than older children.

We can acknowledge the death of the body, says Bering, but we believe that the mind continues: "We have this unshakeable sense that our minds are immortal." Bloom notes that this kind of belief "is universal. You won't find a community anywhere where most people don't believe that they are separate from their bodies."

Mind or soul?

Such hypotheses seem to make intuitive sense. But critics such as Paul Harris of Harvard University say that children learn about the afterlife from others. Working in Spain and Madagascar, Harris and colleagues did studies somewhat similar to Bering's, asking children about the physical and psychological states of a person who had died. Older children and adults were more likely than younger children to think that psychological states continued after death, suggesting that ideas of the afterlife are learned. What's more, people in many cultures distinguish between the mind, which learns and changes over time, and something like an unchangeable soul, says Harris. "To say that there is a continuance of mind after death misrepresents these people's beliefs," he says. "I think people are disposed not to dualism but to 'triadism' of mind, body, and soul."

Even those who embrace the cognitive model concede that more studies are needed to distinguish what is learned from what is innate. As for hypersensitive agency detec-

tion, "it's a compelling idea, but I haven't seen lots of empirical evidence that you can get from there to religious beliefs," says social psychologist Ara Norenzayan of the University of British Columbia, Vancouver, in Canada.

Indeed, even if more data are forthcoming, such models are a long way from explaining the complex systems of gods and rituals that make up religion. Cognitive researchers face what has come to be called the "Mickey Mouse" problem: The Disney character Mickey Mouse has supernatural powers, but no one worships or would

signal that a religion's members are strongly committed to the group and so will not seek a free ride, a perennial problem in cooperative groups (*Science*, 4 September, p. 1196).

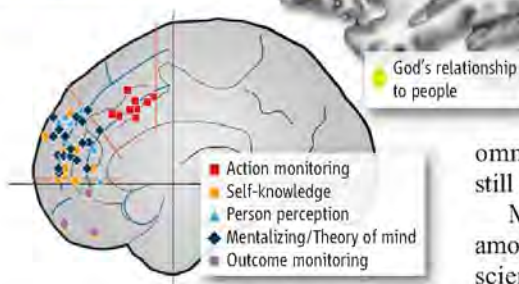
Norenzayan and others also note that helpful behavior is more common when people think that they are being watched, so a supernatural god concerned with morality could encourage helpful behaviors, especially in large groups where anonymity is possible. Some researchers suggest that cognitive tendencies led to religion, which then took hold and spread because it raised fitness.

But others, such as Boyer, counter that this adaptationist explanation is itself light on data. "It is often said that religion encourages or prescribes solidarity within the group, but we need evidence that people actually follow [their religion's] recommendations," says Boyer. "The case is still open."

Meanwhile, disciplinary gaps persist among archaeology, psychology, and neuroscience. Cognitive types insist that ancient objects can answer only a small subset of questions, while some archaeologists dismiss the cognitive model as speculation. Yet there have been some stirrings of interdisciplinary activity. Archaeologist Steven Mithen of the University of Reading in the United Kingdom has suggested that the half-human, half-animal paintings and carvings of the Paleolithic demonstrate that early *Homo sapiens* were applying theory of mind to other animals 30,000 years ago. And anthropologists focusing on the development of religion are finding signs of key changes in ritual at archaeological sites like Catalhöyük. All agree that the field is experiencing a surge of interest, with perhaps the best yet to come. "In the next 10 to 15 years there's likely to be quite a transformation, with a lot more evidence, to give us a compelling story about how religion arose," says Norenzayan.

—ELIZABETH CULOTTA

THINKING ABOUT GOD



Social circuits. When subjects in an fMRI scanner thought about God's relationship with humans, a part of the brain involved in understanding the thoughts of others lit up (top right).

fight—or kill—for him. Our social brains may help explain why children the world over are attracted to talking teacups, but religion is much more than that. "Deriving belief from the architecture of the mind is necessary but not sufficient," says Norenzayan.

He favors an additional class of explanations for why religion is so prominent in every culture: It promotes cooperative behavior among strangers and so creates stable groups (*Science*, 3 October 2008, p. 58). Other researchers hypothesize that religion is actually adaptive: By encouraging helpful behavior, religious groups boost the biological survival and reproduction of their members. Adhering to strict behavioral rules may

Aufbau Ost: Max Planck's East German Experiment

The Max Planck Society's expansion into the former East Germany seeded top science into the region, but challenges remain in making sure the successes take root

In September and October 1989, thousands of East Germans gathered each Monday evening in the city of Leipzig, chanting, "We are the people!" in peaceful protests against their government. The so-called Monday demonstrations were central to the popular movement that toppled the East German dictatorship, which 20 years ago this week opened its borders and checkpoints in the divided city of Berlin, allowing its citizens to travel west. Today, Leipzig is home to cutting-edge research into what it means to be human. Scientists at the city's Max Planck Institute for Evolutionary Anthropology and its Max Planck Institute for Human Cognitive and Brain Sciences are at the forefront of probing how the human mind evolved and works.

When those courageous protesters filled the city streets, Leipzig was a scientific backwater. So how did it achieve its current reputation for research excellence? It's one of the fruits of an ambitious effort by the then-West German Max Planck Society to seed topflight research institutes throughout formerly communist East Germany. Known as *Aufbau Ost*—the phrase means "building up the East"—the project resulted in 20 new outposts of the society within 7 years, several in fields that had been dormant in Germany for a generation. "It was a chance to do something completely new

and to be courageous" in picking research areas, says Peter Gruss, the current Max Planck Society president.

A decade after the last "Eastern" Max Planck institute was founded, the region draws scientists from around the world to study topics as diverse as human evolution, gravitational physics, and the way cells become organs. Observers agree that the society's expansion has been a success—in that it attracted top-class research to eastern Germany. Within the Max Planck Society, there are no noticeable differences between east and west, says Jonathan Gershenzon, director at the Max Planck Institute for Chemical Ecology in Jena. And he has no trouble recruiting talent, he says: "Students are moving from west to east without any concern. People come—there's no hesitation. In that sense, the wall really has been torn down."

But the success of Max Planck's *Aufbau Ost* wasn't quick and easy, and it isn't complete. Some of the new institutes struggled for years, par-

ticularly those burdened with researchers inherited from the East German system or with founding directors who didn't click. And the overall influence of these research oases hasn't yet spread as far as some had hoped. The region's universities, which after reunification underwent a more gradual reformation than the East German Academies, still lag behind their western counterparts. And eastern Germany's industrial base, which largely started from scratch 2 decades ago, still

struggles. "Science is really successful when researchers can see their research reflected in the regional economy," says Hans-Peter Hiepe, who administers the German science ministry's programs for the former East.

Growing pains

In October 1990, less than a year after the Berlin Wall opened, East and West Germany officially reunited. That marked the start of a swift and fundamental transformation of East Germany's research landscape. Under the unification agreement, the West German Council of Science and Humanities (an official advisory body that evaluates the country's research and higher education systems) undertook a rigorous evaluation of the East German Academy of Sciences, which administered dozens of institutes across the sciences and humanities. Within a year, eastern institutes were dissolved, renamed, or often "fileted" and divided between different West German funding organizations. There was also a short-term effort to support top East German scientists (see sidebar, p. 791).

The Max Planck Society, however, was able

Breakthrough. The opening of the Berlin Wall meant dramatic changes for East German research.



CREDIT: TONY STODART/GETTY IMAGES



Human power. Hundreds of thousands gathered in Leipzig's famous "Monday demonstrations," which helped topple the East German regime. Today the city is a major center of research.

to take a longer view. Formed in Germany in 1948, it was West Germany's premier science funding agency after World War II. By 1989, it was running 60 institutes, each led by a handful of directors who receive lifetime appointments and steady funding. The society receives a government-allocated budget but retains the freedom to set its own research agenda, using working groups of current directors to come up with ideas for new institutes.

After the 1989 revolution, initial proposals suggested that the society should thin out its ranks in West Germany to help populate the East, says Andreas Trepte, who helped coordinate Max Planck's Aufbau Ost programs and today works with the society's outreach programs. (A resident of Leipzig in 1989, Trepte participated in the Monday demonstrations.) But the society's president at the time, Hans Zacher, convinced politicians that the current level of research funding in West Germany should be a benchmark—and that the society should expand in proportion to the reunited country's new population.

Keeping with Max Planck tradition, existing directors proposed and debated the focus or theme of each new institute. Working groups looked for areas in which German research was weak, such as anthropology and earth sciences, "or at the absolute interface bridging disciplines," says Gruss, who at the time was a director at the Max Planck Institute for Biophysical Chemistry in Göttingen. Within 7 years, the East had 18 Max Planck institutes, one subinstitute, and one research unit. The society did receive 5% budget increases for nearly a decade to fund the expansion, but it still meant significant belt-tightening in the West, where institutes faced flat budgets for several years and four were ultimately closed.

The first two eastern society outposts—the Institute of Microstructure Physics in Halle and the Institute of Colloids and Interfaces in

Berlin—were started in 1991. Both took over researchers and topics from institutes in the East German Academy of Sciences. The Council of Science and Humanities in its evaluation had found that the East German researchers there had enough potential to join the Max Planck ranks.

Taking them aboard wasn't painless, says Helmuth Möhwald, one of the original directors at the Colloids and Interfaces center. Although the academy scientists were doing fine work, they often didn't fit into the research plans of the new directors, he

says. In his department of 130, "there were at least 30 or 40 who didn't belong but had to be kept working somehow; ... there were too many to simply let them all go," Möhwald says. To make matters worse, the institute was housed in antiquated labs and was split between two locations in East Berlin. The early years "were the worst professional time of my life," Möhwald says, and he often regretted taking the job.

Gradually, Möhwald recalls, he and his imported colleagues found "soft solutions" as they trimmed their staff. "Not more than two out of 100 ended up jobless," he says. The original academy scientists who stayed, he says, were well-trained and highly motivated, and the early hardships started to pay off. In 1999, the institute moved to a state-of-the-art building in Golm, a village just outside Potsdam. When the Council of Science and Humanities evaluated chemistry departments at universities and institutes across Germany in 2007, the departments in Golm were among a handful that received top marks. And earlier this year, Möhwald's fel-

low director, Markus Antonietti, received a prestigious Advanced Grant from the European Research Council—one of just two dozen German researchers to do so.

New starts

The directors who started Max Planck Society institutes from scratch had an easier time. "It is one of the most fantastic things that has happened in my life," says anthropologist Jean-Jacques Hublin, a director at the Max Planck Institute for Evolutionary Anthropology, which began work in 1997. "Building a department up from scratch, the way you want, with the techniques and people you've only dreamed of—it is an extremely rare chance in science." Another director at the institute, linguist Bernard Comrie, agrees. "This was a once-in-a-lifetime offer," he says.

That particular Leipzig institute was an unusual chance for the Max Planck Society as well. For more than half a century, anthropology had largely been a taboo subject in Germany. Until the 1930s, Germany produced some of the world's most influential anthropologists, says Hublin, but "the Nazi regime and the World War almost completely killed the field." It's risen from the ashes in Leipzig. After just a few years, the institute, which now employs nearly 500 people, "has been called the mecca of evolutionary anthropology by some of our colleagues," Hublin says.

Hublin's new scientific home in Leipzig (he was recruited from France) was also an experiment in interdisciplinary research. The five directors—Hublin, Comrie, primatologist Christophe Boesch, psychologist Michael Tomasello, and molecular geneticist Svante Pääbo—come from very different fields, but together they have managed to build a coherent institute. The resulting mix of ideas has led to unexpected projects, Pääbo says, such as looking for the genetic basis of domestication in dogs and rats. "If I had been at an institute of molecular genetics, these are not things I would have been exposed to," he says.

None of the directors at the Evolutionary Anthropology institute is a German citizen. That's not unusual among the Aufbau Ost institutes; nearly two-thirds of the society's directors in the East come from outside Germany. In some cases, such as evolutionary anthropology, Germany had few experts to choose from.

Often, however, foreigners were less intimidated than West German scientists by the so-called Wild East, with its gray, crumbling cities and its society undergoing a wrenching transition from a repressive socialist regime to a democracy with a market-driven economy. "This was the first time in history that a Max

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S Beyond Germany. The fall of the Berlin Wall is the most potent symbol of the revolutionary events that took place in 1989 in the former Eastern bloc, but Europe's transformation went far beyond Germany's borders. That year's downfall of the dictator Nicolae Ceaușescu, for example, brought tremendous change to Romania—and to its research landscape (*Science*, 21 November 2008, p. 1184). In addition to the stories here on Germany, *Science Careers* takes a look online at current science opportunities in Eastern Europe, profiling young researchers and examining programs that promote cooperation between the economically challenged region and wealthier counterparts in Western Europe and throughout the world.

MAX PLANCK
RESEARCH
INSTITUTES

Before and after. The Max Planck Institute for Infection Biology (left) stands just meters from the former path of the wall (right) in Berlin. The Max Planck Society spent €530 million on building new institutes in the East.

AUFBAU OST INSTITUTES	Location	Founding	Employees†
Microstructure Physics	Halle (Saale)	1991	101
Colloids and Interfaces	Potsdam-Golm	1991	238
Infection Biology	Berlin	1992	264
Molecular Plant Physiology	Potsdam	1992	391
Economics	Jena	1992	77
Physics of Complex Systems	Dresden	1992	109
History of Science	Berlin	1993	121
Human Cognitive and Brain Sciences	Leipzig	1993	226
Gravitational Physics	Golm	1994	138
Plasma Physics**	Greifswald	1994	453
Chemical Physics of Solids	Dresden	1995	180
Mathematics in the Sciences	Leipzig	1995	157
Demographic Research	Rostock	1996	151
Chemical Ecology	Jena	1996	316
Dynamics of Complex Technical Systems	Magdeburg	1996	222
Enzymology of Protein Folding*	Halle/Saale	1996	69
Biogeochemistry	Jena	1997	162
Molecular Cell Biology and Genetics	Dresden	1997	361
Evolutionary Anthropology	Leipzig	1997	420
Social Anthropology	Halle/Saale	1998	161

*Research Unit **Branch Institute †In 2007

Planck offer wasn't as attractive for Germans" as for outsiders, says American ecologist Ian Baldwin, director at the Max Planck Institute for Chemical Ecology in Jena.

When Baldwin and another American, biochemist Gershenzon, came to Germany for their recruiting visit, their hosts allowed them "barely 24 hours" in Jena, Gershenzon says. "What I only later realized was that the people who showed us around—West German scientists—would not have taken the job we were being offered." Once back in Tübingen, in Germany's prosperous southwest, their hosts relaxed visibly. "Over a beer one evening they said, 'You know, in 30 years Jena could look a bit like Tübingen,'" Gershenzon laughs. "But that wasn't what we were looking for." To him, moving to Jena was exotic and exciting instead of daunting.

That excitement carried over into the scientists' work. "In the first weeks I was here, I almost couldn't sleep," says Hublin. "I would often go in at 4 a.m. There was so much excitement I couldn't stay still."

The chemistry didn't always work as well as it did in Leipzig. At the Institute for Biogeochemistry in Jena, also founded in 1997, two of the three founding directors had left by 2004, along with three other professor-

level scientists. Personal strains proved to be too much, especially for two-scientist couples for whom the only local career option was to work in the same institute, with one spouse supervising the other. And disagreements between directors didn't help. "There was increasing friction in the whole organization of the institute," says ecologist Ernst-Detlef Schulze, the one founding director who remained. He says the former East German location isn't to blame, however. "The whole thing would have crashed in the West, too," he says. Originally, there had been plans to have as many as five directors, he says, "but that did not materialize. We simply had too many internal problems. ... We were set back, kept busy refilling positions."

The institute, most agree, is now on firmer footing. "It's an example of an institute that needed two starts," says Baldwin, who observed the difficulties up close while the two Jena institutes shared a building as new labs were under construction. "A lot of times it works, but when that special chemistry doesn't occur, it can go sour." The growing pains were something new for the Max Planck Society, Baldwin says. "It was the first time in the history of the Max Planck that people wanted to leave the society," he says. Given that the

organization hired nearly 60 directors in less than a decade, "if something like that hadn't happened, you would almost have to say that the Max Planck didn't take enough risks," says Wilhelm Krull, secretary general of the Volkswagen Foundation in Hannover, who worked at the German Science Council from 1987 until 1993 and at Max Planck headquarters from 1993 until 1995.

Heidelberg East

On balance, Krull says, the brand-new institutes in the former East Germany have panned out well: "You can see that in some areas, starting from scratch and setting up something with an international reputation really helped to put these spots on the map."

One of those bright spots, Krull says, is Dresden. The city, once dubbed "the valley of the clueless" because it was cut off from West German television and radio signals, is now the leading research center in the former East. It is home to three new Max Plancks: the Institute for Chemical Physics of Solids, the Institute for the Physics of Complex Systems, and the Institute of Molecular Cell Biology and Genetics. The latter lured four researchers from Heidelberg, home to one of Germany's best universities and the European Molecular Biology Lab-

oratory, to start over in Dresden. The four directors from Heidelberg—Kai Simons, Wieland Huttner, Marino Zerial, and Anthony Hyman—along with a fifth director, Jonathan Howard of the University of Washington, Seattle, decided to forgo having “departments” of scientists who answered to them and instead gave younger colleagues the chance to help guide the institute. The model, a significant departure from the traditional Max Planck setup, has worked well, Simons says.

“The real question mark in our equation when we moved,” Simons notes, “was could we recruit Ph.D. students and postdocs?” Less than a decade later, the answer seems to be yes. This year, the institute was named the top place to work as a biology postdoc in an international survey commissioned by *The Scientist* magazine. “Within a few years, they’ve managed to make Dresden almost like Heidelberg,” says Krull.

Dresden may also be the clearest success story in terms of Aufbau Ost institutes having a spinoff effect on the region. The Max Planck institutes there are now surrounded by several related research institutes, and more than two dozen technology start-ups have sprung up in the region. The society’s institutes have served as a “crystallization core,” says Gruss. “It’s a wonderful example of how science can drive business.” In the country’s recent Excellence Initiative, which offered universities a chance to get extra funding in a bid to boost a few to world-class status (*Science*, 20 October 2006, p. 400), the Dresden University of Technology received funding for two of its projects, both cooperative efforts with the neighboring Max Planck institutes. It was the only university in the former East outside of Berlin to do as well.

Cooperation counts

In western Germany, relations between Max Planck and universities have often been strained, frequently characterized by rivalry. That hasn’t been the case with the new institutes in Dresden and elsewhere. “It is something that is very striking in Leipzig compared to Munich,” says Pääbo, who was a professor at Ludwig Maximilian University of Munich before moving to Leipzig. “Here we are much more cooperative and open with the university.”

The good relations are partly the result of the society’s overall effort to repair historical rifts. In 1999, it began a new program of International Max Planck Research Schools throughout Germany. Students in the interdisciplinary graduate programs study at the cooperating university and can do research at a Max Planck institute. “It makes it a whole lot easier to integrate the institutes with the university and get away from the castle concept

Why So Few East German Directors?

When the Max Planck Society planted institutes across the former East Germany, it recruited scientists from around the world for its ambitious Aufbau Ost project (see main text). But only two out of more than 60 directors in the newly founded institutes were recruited from the East itself. Today, the society has 267 active directors; only five grew up on the eastern side of the divided Germany. And only one started a career before 1989.

Those statistics are a sign of the mixed blessings that reunification brought for East German scientists. For many, especially the younger ones, it was a great opportunity (see p. 792). But others were set adrift when entire preexisting eastern institutes were closed or cut to a fraction of their original size. Universities, dominated by the Communist Party, were reorganized, and researchers had to compete with West Germans.

As Max Planck undertook its expansion, it was clear that even the best East German researchers simply weren’t in a position to head the new institutes, says Andreas Trepte of the Max Planck Society. “The development of a director needs time, and it was unusual to find someone who was in a leading position in research who had not been involved in politics” and associated in some way with the Communist Party, he says. Those who had stayed out of the party had faced severe career hurdles, Trepte notes, “so no one had the profile” as a leader that was needed to be an institute director.

To support top East German scientists and give the region’s higher education an early boost, the Max Planck Society started a short-term program in November 1990 that gave 28 selected investigators 5 years of funding and administrative support to work at a host university. The university had to agree to hire the researchers as regular professors together with their research teams by the end of the 5-year term.

“It was the best thing that could happen to a scientist from the East at that time,” says chemist Joachim Sauer, who led one of the Max Planck research groups and today is a professor at Humboldt University of Berlin. One of the 28 research groups, headed by biochemist Gunter Fischer, was eventually made into the Max Planck Research Unit for Enzymology of Protein Folding, which today employs 60 scientists in Halle. Today, Fischer is the lone active Max Planck director who had been a professor in East Germany. Four other directors grew up in the East, but they were all graduate students or postdocs when the wall fell.

“There is simply a generation who had already spent too much of their career in the GDR [German Democratic Republic],” says Trepte. Sauer agrees. “I don’t think anyone was overlooked” in the search for new directors, he says. Trepte predicts that the proportion of “Eastern” directors will steadily increase in the coming years. “But really,” he adds, “no one is paying attention anymore” to whether someone comes from east or west of the former wall. That, perhaps, is one of the best signs that Max Planck’s Aufbau Ost has been a success.

—G.V.



Extra boost. Chemist Joachim Sauer received funding as leader of a Max Planck Research Group after German reunification.

that had led to the jealousies and animosities” common in the west, Baldwin says.

However, the relatively weak showing of eastern universities overall in Germany’s Excellence Initiative worries many observers. Although the new Max Planck institutes themselves are producing good science, the overall research landscape around them is not yet strong enough to stand on its own, says Hiepe. “It is as though you’ve just moved into a beautiful new house, the envy of all the neighbors. But soon you discover that the foundation is made of matchsticks,” he says.

“There is still a lot to be done to make eastern Germany more competitive,” Krull agrees. Most important, he says, the universities “need extra money, resources, and infrastructure.” And time is running short, Hiepe says. The eastern states within Germany have received extra infrastructure funding from the

European Union for 2 decades, but that runs out in 2013. And the German “solidarity pact” that has transferred billions of tax dollars to development projects in the East will also phase out by 2019. The research institutes and the universities of the region have to continue to pull together, Hiepe says. The institutes “have to realize that their future is dependent on the university and local companies also doing well,” he says.

Schulze, for one, is in the East for the long term. He retired in September, but he and his wife will stay in Jena, he says. His three children, who grew up in West Germany, all migrated east with their parents. “My son decided to study engineering in Dresden, my elder daughter has a job in Potsdam, and the other has a job in Jena,” he says. “We are very happy to be here. We won’t move back to the west.”

—GRETCHEN VOGEL



PROFILE: HÜBNER FAMILY

Big Dreams Come True

An East Germany family of scientists reflects on life before and after 1989

On 9 November 1989, 23-year-old Dorothee Hübner gathered with her family around their television in the East German city of Halle. As a brand-new biochemistry doctoral student, she was following in the footsteps of her father, Gerhard, and mother, Gertraude, both trained as biochemists at Martin Luther University of Halle-Wittenberg.

Watching crowds of East Germans flood across the Berlin Wall that night, the daughter realized that her life was going to be very different from that of her parents, who had spent decades struggling to do research in East Germany without compromising their personal ideals with allegiance to the ruling Communist Party. "It wasn't until the wall came down that we thought, 'Are we allowed to leave and finally be free?'" she says.

Indeed, the young biochemist soon left Halle, marrying (and becoming Dorothee Kern), settling in the United States, and thriving outside of Germany's rigidly hierarchical scientific establishment, which persisted even after the wall fell. She successfully juggled her career and family life. Dorothee and her husband, now a scientist at pharmaceutical giant AstraZeneca, had two daughters, yet by 1998 she had also become a professor at Brandeis University in Waltham, Massachusetts, and subsequently landed a prestigious Howard Hughes Medical Institute position.

Her parents pursued their newfound freedom at home—as did, eventually, her younger brother Christian, who, after taking positions outside the country, became a physicist in Germany. Taken together, that family watching television in 1989 has become a dynamic demonstration of how the lives of scientists in the former East Germany have changed over the past 2 decades.

Saying no to the Stasi

Before 1989, science in the German Democratic Republic, like almost everything else, was political. Everything from university admissions to teaching positions depended on allegiance to the Communist Party. Ideology also affected the substance of academic work, especially in disciplines such as history, linguistics, and political science. "There was a big difference between the natural sciences and the humanities because the humanities and social sciences were part of the legitimacy and ideology of the Communist worldview," says Dieter Hoffmann of the Max Planck Institute for the History of Science in Berlin.

Joining the party was a prerequisite for advancement; researchers felt pressure to collaborate with the Stasi, the secret police, as well. "In science, many persons were asked from the Stasi to work for them," says enzymologist Gunter Fischer, who was a

◀ **Meet the Hübners.** Georg, Gerhard, Christian, Gertraude, Dorothee (l-r) in East Germany.

colleague of Gerhard Hübner's at the time and is now at the Martin Luther University Halle-Wittenberg.

It was a difficult offer to refuse. In exchange for signing a loyalty oath and an agreement to report back to the Stasi on friends and colleagues, you could attend international conferences and have your career fast-tracked, Fischer says. "If you said no, you'd have no higher-ranking position or travel, and you might lose your job," he notes. The pressure went beyond career and travel to petty indignities. Party members were given the best lab times. Non-party members could only use equipment between 1 a.m. and 3 a.m., Gerhard recalls.

Committed Christians, the Hübners refused to give in; they surrounded themselves with like-minded friends and colleagues. "My parents were never hiding what they were thinking about the whole system," Dorothee remembers. "We knew scientists who were honest and didn't join the party and sell their soul just to have advantages."

After the wall fell, records emerged showing that the Stasi was planning to force Gerhard Hübner and Fischer out of their university jobs; disgruntled party members claimed the two biochemists were "treating the lab equipment as their private property." Asked about the incident today, Gerhard shrugs. "The 'comrades' weren't the best students," he says. "We didn't want them to break it. It was really hard to get spare parts."

Although their supervisor was prominent enough to protect the people in his lab regardless of their politics, Gerhard's official position was scientific assistant (*oberassistent*). His wife wasn't able to get a university position at all. Instead, their adviser found her a job in industry, coming up with ersatz chemical solutions to East Germany's food production problems: artificially aging schnapps and making fake caviar. Professorships and teaching positions were restricted to members of the party. Nonconformity had other implications. "You couldn't speak your mind," Gerhard says. "There was always the fear that you could say something that could have harmed your spouse or kids by accident."

For the three Hübner children, their parent's politics meant a struggle to get into university. The oldest son, Georg, now a chief doctor at a hospital in Germany, secured a university slot. Local officials made it clear that the prospects for Dorothee and her brother Christian were much dimmer. "There was always this goal to bring children from

CREDIT: COURTESY OF DOROTHEE KERN

the working class into the university, not children with academic parents," Christian says. "Of course, if academic parents belonged to the party, somehow they still counted as part of the working class."

Sports ultimately gave Dorothee her ticket to higher education. After a special sports school recruited their athletic daughter as a fifth grader, the parents made a calculated move to keep her in regular school, steering her away from swimming and into basketball. Swimming and other sports where East Germany had a chance at Olympic success were under high pressure to produce results. It was common for coaches to press their athletes to dope. In contrast, sports such as basketball were considered hopeless as far as Olympic medals went.

Dorothee's basketball prowess earned her a spot in high school and later at university. She ended up playing point guard on the East German national team, a position that suited her personality. "I like to be in charge of the game," she says. By 1989, she was the national team captain and a graduate student at Halle.

Dorothee faced a future similar to her parent's lives: scientific isolation behind the Iron Curtain, experimenting for the love of it. For scientists, East Germany was one of the most restrictive countries in the Soviet bloc, notes Hoffmann. Publishing outside the country required special permission.

Like most of the Communist bloc, East Germany was in a perpetual state of financial crisis. Sophisticated lab equipment—such as the nuclear magnetic resonance (NMR) scanners Gerhard and later his daughter needed for their work on protein folding and enzymes—was hard to come by. Biophysical chemist Sture Forsén, who ran a lab in Lund, Sweden, in the 1980s and '90s and is now the director of the Pufendorf Institute at the University of Lund, remembers encountering researchers from behind the Iron Curtain. "There were many good scientists," Forsén says. "The problem was they didn't have proper equipment available and couldn't travel. They were like a kid standing outside a candy store with big eyes, looking in."

Dorothee and her father both note that the situation forced the best East German scientists to be more creative and improvise. The scarcity of resources also influenced what people learned. "The theoretical education, honestly, was better than in West Germany. It was stricter, the pressure was higher, and there were

fewer students," Dorothee says. "Our basic knowledge was on a very high level."

With no pressure to compete for grants or to get into the most prestigious journals, scientists pursued ideas and experiments that might not have been given funding in the West. Dorothee is convinced that the experiments that she is best known for today—including unusual approaches to measuring the way protein structures shift and change, cutting across the fields of chemistry, physics, and biology—are rooted in this improvisational spirit.

Still, Dorothee readily acknowledges that the end of communism changed everything. "It couldn't have come at a better time," she



"It was my dream since I was a little kid to go to America, study science, and play basketball. It's always good to have big dreams."

—DOROTHEE KERN,
BRANDEIS UNIVERSITY

says. "I couldn't go any further without having a better NMR machine." When the wall fell just a month into her Ph.D., she set out to look for labs that would let her follow her interest in protein folding; she ended up approaching Forsén with big eyes.

In 1990, Dorothee began commuting by train and ferry from Halle to Lund to conduct experiments in Forsén's lab there. She would spend 4 years juggling her Ph.D. research in two countries with competitive basketball on the unified German national team and Halle's local team. Her energy made an impression on colleagues. "She was bouncing around the lab all the time," Forsén remembers. "She's not very tall, but she was made captain of the national team—that tells you something of her qualities."

Empowered by her new freedom, Dorothee next moved on to a postdoc in the United States before landing at Brandeis.

Already a young mother, she found the American academic world more accepting of women scientists. "The attitude in Germany was either have a family or do science," she says. "I came over here and it was the opposite. It's really a blessing."

After the fall

Many weren't as nimble at navigating the transition. The fall of the wall turned East German science on its head. The Stasi archives were opened in 1991, revealing that some of the country's top scientists had been collaborators and forcing them out of universities. In the social sciences, entire institutes were simply closed, their scholarship too tainted by ideology to salvage. And after decades of isolation, an entire generation of scientists suddenly had to compete for jobs with West Germans and others. Gerhard found himself the lone East German in the running for a position as the chair of his department, up against more than 30 West Germans—he won.

The country's reunification did bring the Hübner family an unexpected benefit. Dorothee met her future husband at the first combined meeting of the East and West German biochemical societies. Her first paper in *Science*, a 1997 report on how vitamin B-1 is activated in enzymes, was a family affair, with her husband and father as co-authors.

Dorothee's collaborations now continue with Christian, who back in 1989 had just entered university at Halle, where he studied physics. He remembers the years after the end of communism as chaotic and distracting. "For the first few years of my studies, the old order had gone and there was no new order," he says. Christian worked for a local parliamentary candidate's campaign and might have left science if his boss had won a seat in 1994. Instead, he went on to study in the United States and Switzerland, returning to Halle as a professor in 2003. When the University of Lübeck made an offer Halle couldn't match—head of its physics institute—he accepted. Christian recently co-authored a paper in *Nature* with his sister, helping her team dissect the dynamics of a single molecule in real time.

Like many of their East German contemporaries in the 1980s, the Hübner family never expected the system to change. Yet deep inside, Dorothee says she had always held out hope. "It was my dream since I was a little kid to go to America, study science, and play basketball," she says. "It's always good to have big dreams."

—ANDREW CURRY

Andrew Curry is a freelance writer based in Berlin.

DNA SEQUENCING

No Genome Left Behind

A project to sequence 10,000 vertebrates has just been launched, but sequencing technologies are not yet up to the task

Times have changed. A decade ago, it took several rooms full of sequencing machines and millions of dollars to decipher the genome of a small nematode. This month, two sequencing machines and \$500,000 yielded a draft of the far more complex DNA of the cod. Now, a group of genome and museum experts are calling for the unraveling of 10,000 vertebrate genomes, about one per genus across the backbone animal world. They have already set in motion a global effort to gather the DNA needed for such an undertaking. "This is the most comprehensive experiment that anyone has ever proposed to analyze the evolution of genomes," says Oliver Ryder, a conservation biologist at the San Diego Zoo in California.

The Genome 10K plan, formally announced this week, is short on details: where funding will come from; what sequencing strategy to use; and how to process and make use of the data generated. But supporters are gung ho. "It's a grand plan that will happen," predicts Joel Cracraft, an ornithologist at the American Museum of Natural History in New York City.

New sequencing technologies aided the rapid completion of the cod genome project, one of about a half-dozen efforts using advanced, low-cost methods to analyze large

vertebrate genomes, originally considered too complex to be unraveled with the newer technologies. And better tools are coming online. Besides, says Byrappa Venkatesh of the Institute of Molecular and Cell Biology in Singapore, there's a precedent for being impetuous: "After all, when the Human Genome Project was launched, there was neither an appropriate technology for sequencing a complex genome nor a suitable algorithm for assembling and annotating" the results.

Beyond the human genome

Ever since they finished the human genome sequence, researchers have been champing at the bit to sequence other genomes to compare with human DNA. Such comparisons help identify conserved regions that likely serve key roles in survival and also regions that differ between species and likely represent adaptations to a particular way of life.

With that in mind, the National Human Genome Research Institute (NHGRI) 5 years ago began to assemble a list of 32 mammals and 24 other vertebrates that would make good candidates for analysis. David Haussler of the University of California, Santa Cruz, and Stephen O'Brien of the National Cancer Institute in Frederick,

Maryland, were part of the selection committee. "One of the most difficult things was to get specimens that had good DNA," O'Brien recalls. Anticipating that genome sequencing will get much cheaper, Haussler, O'Brien, and Ryder decided to prepare for a full-out assault on vertebrates.

The three organized a meeting in April at which 50 participants from the United States, Canada, South and Central America, Europe, and Asia came up with a list of 10,000 candidates, one-sixth of the known vertebrates. After an evening of hashing out reservations and concerns, they divided into animal-specific groups to see what suitable DNA existed, and where. To their surprise, they concluded that freezers around the world held DNA from more than 16,000 species.

As described online 5 November in the *Journal of Heredity*, the Genome 10K project has compiled a list of tissue samples from more than 43 institutions. For 600 species, cell lines already exist; the plan calls for establishing cell lines for 2000 more species. In addition, the researchers want to sequence several individuals for at least one species per order.

Advocates say the project will reveal new information about the human genome and basic biology. With dense sampling, "the insights into genome evolution and speciation from an evolutionary perspective would be extremely powerful," says William Murphy of Texas A&M University in College Station. Cracraft, for example, is curious about grebes and flamingos, which look nothing alike. "The question is how to account for that disparity in biology and form," says Cracraft.

Molecular menagerie. These animals and thousands of other vertebrates may one day have their genomes sequenced.



"Whole genomes are really going to spur things on."

Others believe the endeavor is essential to aid conservation efforts. "Wait another 20 years and it will be too late for several species," says Olivier Hanotte, a conservation biologist at the University of Nottingham in the United Kingdom, who has pushed to have endangered species included on the list.

Sequencing challenges

But waiting might be a good idea. So-called next-generation technologies have revolutionized sequencing during the past few years, providing cost and time savings (*Science*, 17 March 2006, p. 1544). But there have been tradeoffs: Efficiency brought a shorter "read length"—the number of consecutive bases that can be sequenced—and lower accuracy for each base determined. The accuracy can be compensated for by sequencing the genome multiple times to detect errors. Short reads aren't much of a problem for human DNA, because the existing reference human genome can help sort out where these bits of sequence belong. But assembling large genomes from scratch is quite a challenge.

Undaunted, about a dozen groups are pushing the limits of the new technologies, taking a variety of approaches, with varying degrees of success. "We're trying to figure out how to best do this," says Richard Wilson, director of the Genome Sequencing Center at Washington University School of Medicine in St. Louis, Missouri. Right now, there's a penalty for using cheaper methods: The least expensive sequencer generates the shortest reads, which are the hardest to assemble. Illumina technology produces a base of sequence for about one-tenth the cost of Roche 454 technology, but 454 has the advantage of generating reads about 350 bases long compared with Illumina's 75 bases.

When they tackled the cod genome in late 2008, Kjetill Jakobsen, a genomicist at the University of Oslo, and his colleagues used Roche 454. It took several months using two machines to generate the bulk of the 750-million-base cod sequence, covering the genome 30 times over. When they were done, "we used brute force," relying on extensive computing power to put the pieces together, says Jakobsen. The price to sequence: \$500,000.

At least two sequencing centers have bet that they can sequence large genomes even with Illumina's very short reads. In January,

the Beijing Genomics Institute in Shenzhen announced that in a month it generated 150 billion bases and stitched them together into a 3-billion-base genome of the Olympic mascot, a panda. They came up with their own computer program for assembling the genome, and the draft consisted of thousands of pieces averaging 300,000 bases long.

The Broad Institute in Cambridge, Massachusetts, has assembled the stickleback genome sequence this way and is now working on the bush baby. "We've believed in this concept for a while, even when people thought it was crazy," says the Broad Institute's Chad Nusbaum, who more than a year ago showed that bacterial and fungal genome sequences were doable with short reads. For large

the company's Stephen Turner. Last February, he reported read lengths of 3000 bases. "We're absolutely going after the de novo assembly market," says Turner.

Wishful thinking?

Even with all the advances, "the technology isn't there yet" for the kind of sequencing envisioned by Genome 10K, says Adam Felsenfeld of NHGRI. O'Brien, Haussler, and Ryder agree. They need the price tag to drop to \$2500 for sequencing a large genome. Their goal is to spend \$50 million for the whole project.

SPECIES ON THE SEQUENCING DOCKET

Group	Known Species	10K Species	Proportion (%)
Birds	9723	5074	52
Reptiles	9002	3297	37
Mammals	5416	1826	34
Amphibians	6570	1760	27
Fish	31564	4246	13
Total	62275	16203	26



Coming up with the cash could be a challenge. Murphy, Haussler, and O'Brien, among others, hope private philanthropists will foot the bill. Hanotte is calling for public funding to ensure the unrestricted access to the data. Venkatesh thinks funding agencies that support biological, conservation, or biodiversity research will chip in. But NHGRI, which has supported much of the sequencing of large genomes in the United States, is noncommittal. "I don't know how much we will see it fitting in with our priorities," says Felsenfeld.

Furthermore, although Felsenfeld and others applaud Genome 10K for identifying tissue sources early, bioinformaticists are worried that insufficient attention has been paid to data management for the assembly and analysis of sequences. To assemble 10,000 genomes in 5 years as proposed will require processing a genome a day, warns Webb Miller, a computer scientist at Pennsylvania State University, State College. "There's a real problem here," he notes.

Despite large gaps in their plan, the Genome 10K leaders are supported by an enthusiastic cadre of peers. Participants at the April meeting and their colleagues are busy checking out the samples and deciding which ones have top priority. "We've got real momentum now," says Haussler. He hopes to do a pilot study demonstrating that these samples are suitable DNA sources for sequencing. Sooner or later, those genomes will be sequenced, Turner predicts. "I don't think it's a question of 'if' but 'when.'"

—ELIZABETH PENNISI

genomes, "it's not solved yet, but there are enough indications that it is going to work that we're convinced this is the way to go."

Several teams are using a mix of sequencing technologies, hoping this hybrid approach will boost accuracy and efficiency. As Washington University School of Medicine starts the vervet monkey, it expects to use both Illumina and 454 machines and to rely on the traditional capillary-sequencing technology used on the human genome for sequencing the ends of 100,000-base bacterial artificial chromosomes representing the monkey's genome. "For mammalian-sized genomes, that's going to be very helpful," says Wilson. Richard Gibbs of Baylor College of Medicine in Houston, Texas, has adopted a similar strategy for the baboon. That hybrid approach can speed up finishing, "but it's not a knock-it-out-of-the-park solution," Gibbs points out. "Difficult sequence is always difficult sequence."

Other potentially revolutionary tools could be on the market in the coming year. For example, the Menlo Park, California-based Pacific Biosciences is working on a technology that tracks the enzyme that puts DNA together, noting each new base added. It can sequence two to five bases per second, says



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LETTERS

edited by Jennifer Sills

Space Goals Require Worldwide Participation

IN HIS NEWS OF THE WEEK STORY "OBAMA FACING TOUGH decision on whether to keep aiming for the Moon" (25 September, p. 1606), A. Lawler states that Norman Augustine's committee concluded that NASA doesn't have enough money to carry out the current plan for astronauts to go to the moon by 2020 and suggested that other destinations and goals be considered. To accomplish a reasonable set of goals beyond low Earth orbit on time scales that will maintain a healthy space program, the United States can no longer afford to go it alone or even call the shots on international cooperation.

Ground-based U.S. astronomers and planetary scientists have already recognized that international participation is necessary for their large, expensive programs (1). Our national space dilemma can be solved by including international partners at all stages of future expensive manned space projects, from planning, to design, to use. Partners should include those now participating in the International



The International Space Station

Space Station (ISS), with future decision-making authority distributed among all organizations in proportion to their financial levels of contribution.

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Note

1. Examples include NSF's Gemini program, NASA's International Space Station, observing complexes of Very Long Baseline Interferometers, and NASA's Moon Mineralogy Mapper (M-cubed) aboard the Indian Space Research Organization's Chandrayaan-1 spacecraft.

Biobanks: Questioning Distinctions

D. GURWITZ *ET AL.* ("CHILDREN AND POPULATION biobanks," Policy Forum, 14 August, p. 818) propose a policy for population biobanks in which samples and data from children will not be accessible to researchers outside the biobank until the children give consent when they reach the legal age to do so. They suggest that this policy be applied to population biobanks but not to disease-specific biobanks. This distinction is relevant from neither a scientific nor a clinical point of view.

A growing body of data shows that health events early in life may affect adolescent and adult health (1, 2). Other empirical studies support the hypothesis that epigenetic changes caused by environmental conditions early in human life can have effects throughout life (3). Because it is likely that genetic epidemiology will uncover more of these

gene-environment interactions, it is essential that scientists with multiple backgrounds and expertise have access to samples and data that are representative of the different phases of life. Moreover, sample collections with different origins should be pooled to validate the biological importance of genes and identify previously unknown causes of disease (4, 5).

The distinction is also difficult to justify, given that balancing potential harms and benefits is different for each type of biobank. The authors do not identify the "fundamentally different" potential harms posed by participation in population research, other than suggesting the possibility of identification from DNA sequences deposited in public databases.

In addition to this false distinction, the authors admit that the suggested policy "may negatively impact research." An alternative to imposing the suggested restrictions on research is to regulate access to genetic

data from all types of biobanks, develop robust data security measures, and criminalize the misuse of genetic information, as has been done recently in the Swedish law on genetic integrity (6).

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Biobanks: Too Long to Wait for Consent

IN THE POLICY FORUM "CHILDREN AND POPULATION biobanks" (14 August, p. 818), D. Gurwitz *et al.* proposed that DNA information from children should not be shared from population databanks until those children reach adulthood and give specific consent. This proposal is flawed.

First, the authors place too much weight on consent. The potential harm posed by data sharing can be mitigated by limiting the data to be shared, removing identifiable elements, and maintaining thorough oversight. BioVU, Vanderbilt's resource of residual blood samples and information from electronic medical records, uses de-identification during data and sample collection and again before sharing data, as well as careful internal and external oversight. Moreover, limited-access research databases are likely to be relatively unattractive sources of personal and genetic information for hackers. Any concerns about the coverage of the Genetic Information Nondiscrimination Act should be addressed through legislation.

Second, the authors suggest that parental permission is an appropriate justification for permitting information from disease-specific databanks to be shared. This justification fails because it falls into the trap of the therapeutic misconception. Therapeutic research involves trying a new intervention, such as a new drug. Sharing DNA information with other investigators is not therapeutic because it is very unlikely to help any particular child in the short term. Their proffered distinction would also undermine the moral acceptability of the National Children's Study, given that the data sharing planned in this population-based study would be deemed unacceptable.

Third, waiting 18 to 21 years to combine data from various sites, an essential step in examining how genetic variation affects the health of children, means that research on children will always lag a generation behind

Reports: "Evidence for obliquity forcing of glacial Termination II" by R. N. Drysdale *et al.* (18 September, p. 1527). In the fourth complete sentence on p. 1529, the word "retreats" should have been deleted. The correct sentence should read, "Under warm conditions, more intensive MOC increases regional SSTs and displaces the polar front to higher latitudes." In addition, the callout of reference 34 on p. 1530 should have been a callout for reference 14. The callout for reference 34 should have appeared on p. 1529 with the callout for reference 32.

This Week in Science: "MITE-y jumps" (11 September, p. 1317). The following sentence was incorrect: "Surprisingly, the element coding for the transposase repressed its own movement unless the MITE was present." The Report by G. Yang *et al.* (11 September, p. 1391), to which the entry referred, did not reach this conclusion.

Reports: "Water and the oxidation state of subduction zone magmas" by K. A. Kelley and E. Cottrell (31 July, p. 605). Reference 14 appeared incorrectly as "in press." The correct reference is: 14. E. Cottrell, K. A. Kelley, A. T. Lanzirrotti, R. A. Fischer, *Chem. Geol.*, 10.1016/j.chemgeo.2009.08.008 (2009).

and will reflect only the medicine and environment of years gone by.

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Biobanks: Oversight Offers Protection

D. GURWITZ *ET AL.* ("CHILDREN AND POPULATION biobanks," Policy Forum, 14 August, p. 818) are right to argue that the storage and use of tissue samples and extracted DNA from children for genetic research pose specific ethical questions, which are different from those posed by adult samples. We agree that these specific questions are linked with the special vulnerability of children and their gradual growth towards autonomy (1). However, we disagree with their solution that the use of samples and individual DNA sequence from children should await their consent as adults. Such an approach will delay the advancement of important scientific discoveries and run counter to the altruistic reasons for participation in research studies.

Guidelines and recommendations about stored tissue samples, such as those of the World Health Organization, often refer to the concept of minimal risk when discussing the issue of minors (2, 3). It is as of yet uncertain, however, what level of risk is associated with pediatric biobanks and the associated genetic information. Ethics and biobank literature outline the danger that employers, insurers, and other third parties would access such information and would use this to discriminate against the research subject (4). We question how realistic such a scenario is in public health care systems that operate in most European countries.

We believe that as long as collections are properly governed, and policies regarding data protection and use of genetic information are

in place, the risk associated by having DNA stored, extracted, and sequenced in research databases and used by researchers is minimal. Outcomes of biobank research, in the form of association of genes and conditions with certain populations, could lead to group stigmatization and discrimination (1, 5). However, this would affect children of the stigmatized group anyway, regardless of whether they personally contributed to the research or not.

Another risk associated with the use of pediatric stored tissue samples is that the research parents consent to would be in disagreement with the values that the minors hold. In our experience of interviews with teenagers, they trusted the decisions their parents had taken when they were small children, as long as they would gradually be more involved in the decisions when they grew older. A system that gradually puts more and more weight on the assent of minors as they grow older as has been successfully achieved by the Avon Longitudinal Study of Parents and Children (6).

To maintain the right balance between protection of children and advance of scientific discovery, we propose the following solutions: (i) Robust policies and governance structures should be put in place to ensure adequate data protection. Such structures should not only operate on the level of the biobank itself but also in a wider organizational and societal context. (ii) Ethics committee oversight is essential to monitor data protection, ensure that children are not burdened by the research, and review consent when research reaches certain milestones. Parents and children should be able to reconsent when research reaches these milestones. (iii) Genetic research done on children should be done for children as recommended by the CIOMS guidelines (7). This implies that part of a child's genome should not be sequenced unless it is for the sake of studying conditions that may occur or develop in childhood.

Public trust in science is essential to the progress of science. Such trust entails that

Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 3 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

children are properly protected, but also that medical science can proceed and that resources are well used.

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Response

WE AGREE WITH HANSSON AND MASCHKE that early-life events affect adult health. Our question was how to best protect the privacy and autonomy of children. Human genetics knowledge is rapidly changing; policies that were assumed to be sufficient for protecting research participants' privacy and autonomy decades ago are no longer adequate in the age of direct online data access and Web-based personal genomics (1, 2). Scholars now agree that true anonymization cannot be assured (3).

We concur both with Hansson and Maschke and with Brothers and Clayton that ideally the solution must focus on comprehensive legislation that criminalizes the misuse of individual information and not on the alternative—putting further restrictions on genetic research. However, legislation is only part of a solution, and it can be a very slow process: In the United States, the Genetic Information Nondiscrimination Act (GINA) of 2008 took 5 years to enact. Legislation of this sort may be hard to achieve in many countries. Moreover, laws cannot be substitutes for trust or for the responsible conduct of science.

Regarding our policy suggestions for access to DNA samples stored by disease-specific versus population biobanks, both Hansson and Maschke and Brothers and Clayton missed our point. We do not focus on different types or magnitudes of potential harms, as Hansson and Maschke suggest, but on the relevant differences of the situations that may justify incurring the risk of harm. In terms of ethics, the potential harms

and benefits of clinical sample collection for diagnosis and therapy differ substantially from those that arise in the context of population biobanking.

What matters in disease-specific biobanking—or, as Brothers and Clayton call it, therapeutic research—is that the children's DNA samples are being collected in a clinical context; parents may not have given their permission if approached outside this context. Indeed, almost half the mothers surveyed about participating in the KidsGene pediatric biobank in Chicago wrongly assumed that the research was aimed at helping their individual child (4). Alarming, about one-third of pregnant women surveyed about participation in the National Children's Study were unwilling to participate, and refusal was higher among more educated women (5).

Brothers and Clayton are concerned that adopting our suggested policy would mean that "research on children will always lag a generation behind." However, our suggestions included in-house genotyping of children's DNA samples (which may include outsourcing in lieu of local facilities) for retaining control over spread of information and material. Such solutions, the feasibility of which needs further assessment, would assure that research will not be substantially delayed. Moreover, mature minors may reach a level of autonomy sufficient for consent on inclusion in biobanking projects before the age of legal adulthood (6).

Hens *et al.* summarize our proposal incorrectly: It is not the use of samples but their distribution to outside researchers that we propose should await the consent of children. Also, they assume that we are concerned mostly about discrimination by insurance companies. However, other, yet-unforeseen risks arising from uncontrolled distribution of DNA samples could lead to individuals being stigmatized, as in the example of assumed genetic markers for personality traits.

Hens *et al.* suggest alternative protective steps, including a policy "that part of a child's genome should not be sequenced unless it is for the sake of studying conditions that may occur or develop in childhood." Apart from the fact that whole-genome sequencing may soon become the norm in genetic research, we find restricting research topics to be more problematic than our proposal to restrict access. Animal studies show that late-gestation stress may affect adult immune response (7); thus, studies on methylation patterns in children's DNA samples combined with their mothers' pregnancy medical records may illuminate the etiology of adult diseases. We should not prohibit such studies.

Our Policy Forum presents a topical dilemma that needs to be addressed. These Letters and numerous Internet commentaries [e.g., (8–10)] tell us that our key purpose—opening a public discussion—is being well served.

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Research for All Science Teachers

M. SINGER'S EDITORIAL, "GREAT TEACHERS for STEM" (28 August, p. 1047), is right on target. Implementation, however, will require a cultural shift. Although the United States needs more young scientists to go into pre-college science teaching, insufficient numbers will do so if they are obliged to abandon their own adventures in science. As with college professors, we should enable and expect 7th- to 12th-grade science teachers to participate regularly and significantly in scientific research. Sustained research activity, not just workshops or tag-along excursions, should be part of the opportunity and responsibility of a science teacher.

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EVOLUTION

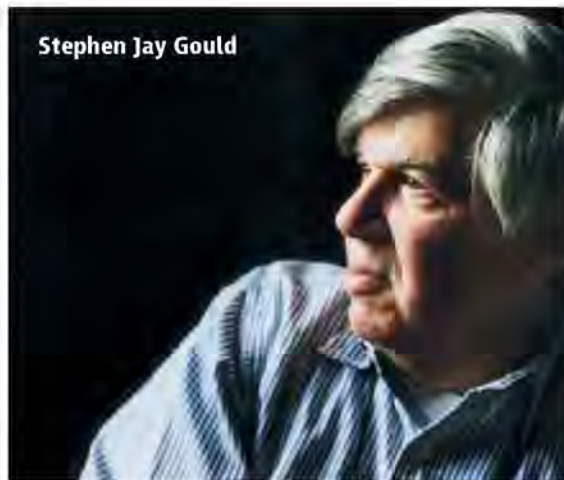
Darwin Is Dead—Long Live Evolution

Vassiliki Betty Smocovitis

Stephen Jay Gould and the Politics of Evolution arrives just in time. Just when it looked like the “ultra-Darwinists” were winning the “year of Darwin” with their interminable love-fests, triumphalist narratives, and self-serving revisionist histories; when we were starting to think that Darwin was the only evolutionist to have lived in the past 150 years; and when we might conclude that nearly the entire evolutionary community had drunk the Kool-Aid of antiquarian Darwinism, David Prindle’s book appears to give us pause. It reminds us of the late paleontologist, the heady days of late 20th-century evolutionary science, and all the political underpinnings of evolutionary biology that Gould was so fond of revealing. No fan of simplistic, reductionistic, ahistorical, or apolitical views of scientific knowledge, Gould offered a distinctly self-critical view of science, one that would likely challenge much of the ultra-orthodoxy passing as reflective history and science written expressly for the year of Darwin.

Gould viewed science from the perspective of a practitioner, while using critical methods from history, philosophy, and social theory to expose the complex scaffolding that had given rise to it. His work frequently revealed the biases and prejudices that were concealed by the kind of historical rewriting that eliminated precisely those very human frailties as well as the sociopolitical forces that often overrode even the most rational of methods. At heart, Gould understood that science was itself a political process—not just in the superficial sense of the “politics of science” (where the politics gets in the way of science) but more that there always is a “knowledge-power” relation. For Gould, history, philosophy, social, and political theories were not just linked with science but were in fact constitutive of science. Such a view did not always sit comfortably with his colleagues (and even Gould himself bore some notable contradictory elements in his simultaneous embrace of science and ideology). That is one reason he drew the ire of so many of his contemporaries who viewed

Stephen Jay Gould



him as inappropriately political for a scientist. This was ironic, indeed, because many of his opponents themselves had equally strong political views (usually in the opposite direction), although those were usually masked by what appeared to be an objective, apolitical view of science (sociobiology, anyone?)

The book begins to explore some of these themes in the works of Gould. The author, a professor of government at the University of Texas, argues that Gould’s mind worked simultaneously in two “parallel tracks,” one scientific, the other political. Interpreting these in terms of a “consistent whole,” Prindle presents his analysis as a history of ideas. He organizes the book thematically around Gould’s writing style, his philosophy of

science, his use of historical inquiry, and his views on the nature-nurture controversy and on science and religion. Prindle concludes with an assessment of Gould’s original contributions to modern evolutionary theory.

Not much in Prindle’s treatment is surprising. The author characterizes Gould as a leftist but refutes the mythology that he was a Marxist. Prindle tells us that Gould developed a “charming style” of writing, which made him both an effective popularizer and a skilled rhetorician, and that he was likely the most “enthusiastic creator of metaphors in the history of science.” Prindle’s analysis does, however, miss the tragedy of Gould’s magnum opus, *The Structure of Evolutionary Theory* (1), a vainglorious attempt to synthesize all of his thoughts on the subject

that ended up as a nearly unreadable 1433-page behemoth. Prindle describes Gould’s use of contingency in evolution and history, how it played out in the fossil record in instances like the celebrated Burgess Shale, and how it could also introduce chance and avoid determinism in evolutionary processes. The author discusses Gould and his “ideological consociate” Richard C. Lewontin’s celebrated critique of the adaptationist program in their “Spandrels of San Marco and the Panglossian Paradigm” paper (2) and the critical responses it provoked.

There is much on Gould’s critique of intelligence testing and on his battles with bugaboos like Richard Herrnstein, Charles Murray, and their notorious book *The Bell Curve* (3), in which they argued that social and economic differences between blacks and whites in the United States were not due to racial discrimination but to differences in intelligence between the two groups. There is also, of course, much on Richard Dawkins, along with discussions of their fundamental differences over selectionism, reductionism, determinism, and what have you. As Prindle correctly explains, Gould’s self-critical perspective on evolution provided fodder for creationists and lacked the consistency of Dawkins’s atheistic position. Gould’s more nuanced, irenic views, however, avoided what he saw as “the polemics of ill-conceived battle between science and religion.”

Prindle is at his best in the chapter “The contours of history.” Here, the author addresses Gould’s deep understanding of the philosophy of history and considers its influence on both his historical writing and his evolutionary science. Prindle’s discussion of what he terms Gould’s “historical empathy” is stunningly insightful, although I wish more had been done with Gould’s historicism. Gould was a radical historicist and sensitive to a number of movements in the humanities that stressed the view that science is a historically rooted and culturally embedded practice. I also appreciated the chapter on human nature, in which Prindle brings to bear his understanding of political theory. One’s politics do, after all, hinge on the fundamental question of human nature. As expected, Prindle discusses Gould and Niles Eldredge’s

Stephen Jay Gould and the Politics of Evolution

by David F. Prindle

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celebrated critique or amendment—depending on one's point of view—known as punctuated equilibrium. But, sadly, there is next to nothing on Gould's insights into development or what is really his finest historical work, *Ontogeny and Phylogeny* (4). Those topics would have been especially timely in the current excitement over the synthesis between evolution and development.

The book suffers from several shortcomings. To begin with, Prindle's account is too brief for a complex figure like Gould. There is also a lack of depth in terms of the technical explication of the science. The style of the writing is overly casual. And Prindle's account demonstrates his unfamiliarity with the history of science. He seems unaware of the vast literature that has explored not only politics and science but also the interplay of science, worldview, and ideology [for example, (5, 6)]—exactly the kind of intellectual history Prindle attempts. His account could have been greatly strengthened had he drawn on such work. It is also weakened by Prindle having largely restricted himself to a selection of Gould's published works (no archives), not all of which he systematically examines.

I am not convinced of Prindle's conclusion that future historians will see Gould's legacy only in terms of the spandrel and the related concept of exaptation—elements of his criticisms of the adaptationist program. Those

seem to be only some of his scientific contributions. Also, as a historian of science, I don't share Prindle's problem with the incomplete nature of Gould's evolutionary (or political) theory. Who in the history of science (or politics) has left behind a fully formed theory? Not Darwin: It took a small army of workers, including geneticists and mathematicians, simply to get to the synthetic theory of evolution. And that was before 1953, when the structure of DNA was determined. Science, like politics, is rarely about completion.

Whatever its drawbacks, *Stephen Jay Gould and the Politics of Evolution* is a welcome addition to our understanding of late 20th-century evolutionary science, of which we know too little, and a provocative introduction to a major figure. The book is especially well-timed to challenge the many ultra-orthodox, ultra-Darwinists who seem to have taken center stage for 2009. It reminds us that evolution is represented by a plurality of voices. The book's final chapter does much to honor the lively spirit of Gould's special blending of politics and science. The opening section of the chapter rolls together 19th-century Darwinism, conservative politics, and references to the egregious misuse of history by the likes of filmmaker Ben Stein (*Expelled*), conservative pundit Ann Coulter, and others who link Darwin to Hitler. Here, Prindle finally gets into the nitty-

gritty unpleasantness of the politics of evolution as manifested in some current populist anti-evolutionist movements. Reading this section reminded me that were Gould still alive (he would have been only 68 this year), he would likely be leading a chorus of people in the fight against the rising tide of such populist critics. Historicizing Darwin and Darwinism, making them truly things of the past, and decoupling Darwinism from modern evolution, he would be helping us move forward into 21st-century evolutionary science. As we enter the home stretch of 2009, let us remember Gould and honor his legacy with the following thought: Darwin is dead. Long live evolution.

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HISTORY OF SCIENCE

Evolution of the End of *Origin*

R. W. D. Nickalls

NOTE BOOK OF 1837

Astronomers might formerly have said that God foreordered each planet to move in its particular destiny. In the same manner God orders each animal created with certain forms in certain countries; but how much more simple and sublime [a] power—let attraction act according to certain law, such are inevitable consequences—let animals be created, then by the fixed laws of generation, such will be their successors. (1)

SKETCH OF 1842

There is a simple grandeur in the view of life with powers of growth, assimilation and reproduction, being originally breathed into matter under one or a few forms, and that whilst this our planet has gone circling on according to fixed laws, and land and water, in a cycle of change, have gone on replacing each other, that from so simple an origin, through the process of gradual selection of infinitesimal changes, endless forms most beautiful and most wonderful have been evolved. (2)

ESSAY OF 1844

There is a simple grandeur in this view of life with its several powers of growth, reproduction and of sensation, having been originally breathed into matter under a few forms, perhaps into only one, and that whilst this planet has gone cycling onwards according to the fixed laws of gravity and whilst land and water have gone on replacing each other—that from so simple an origin, through the selection of infinitesimal varieties, endless forms most beautiful and most wonderful have been evolved. (2)

ON THE ORIGIN OF SPECIES, 1859

There is grandeur in this view of life, with its several powers, having been originally breathed into a few forms or into one; and that, whilst this planet has gone cycling on according to the fixed law of gravity, from so simple a beginning endless forms most beautiful and most wonderful have been, and are being, evolved. (3)

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EDUCATION

Building on No Child Left Behind

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A dominant strand of U.S. educational policy for the past two decades has been incorporation of information about student achievement into management and regulation of schools. Although this policy idea is often attributed simply to the federal No Child Left Behind Act (NCLB), 44 states already had some form of test-based accountability (TBA) when the NCLB came into existence in 2002. NCLB transformed TBA into a national strategy, placed a clear goal on improvements in student achievement as reflected on certain standardized tests, and established a series of actions and penalties for failure to meet annual improvement goals on those tests (including school closure in the worst cases). More than 70% of the American public favors renewal of federal accountability legislation (1), and performance on similar tests is known to relate to important economic outcomes (2). In 2009, the U.S. Supreme Court focused on the importance of outcome accountability in a major school finance decision (3). Thus, TBA has become a fixture of American education. However, it is also clear that the current version could be improved significantly.

What Has Been Accomplished?

NCLB focuses on having all students proficient in reading, math, and science. All states had to develop learning standards and assessments of student performance. Individual schools are required to be on a path toward universal proficiency by 2014. Three lines of inquiry suggest that existing accountability systems have led to larger gains than would be expected in a world without them.

First, comparisons of math and reading performance across states from the National Assessment of Educational Progress (NAEP)—often called the Nation's Report Card—provide some insights. Other things being equal, states introducing accountability earlier showed larger gains on NAEP during the 1990s (4). Moreover, students in states with stronger accountability performed better (5). Students in Florida schools graded "F" for failing state accountability measures, and thus subject to sanctions, showed positive effects of school accountability when compared with similar schools scoring slightly

better at "D" (6). Results of individual state tests over time show that student achievement gains tend to be larger after the introduction of NCLB than before (7). For example, the success of Florida and Texas in raising the achievement of Hispanics and low-achieving students is attributed in part to their use of accountability systems (8–10). Because it is difficult to distinguish impacts of different simultaneous reforms and to establish causation, some uncertainty remains, but the combined picture shows improved student performance after introduction of TBA.

Second, accountability, particularly after NCLB, focused attention on achievement of disadvantaged populations. Evidence indicates this has changed the dynamic within schools, yielding improvements in previously low performing groups (4, 8, 7). In aggregate terms, for example, the black-white gap in mathematics achievement (measured by NAEP for 9- and 13-year-olds) significantly closed between 1999 and 2007 (11).

Third, U.S. evidence is consistent with a growing body of international evidence pointing to the value of central exit exams and more regular accountability. Particularly where there is more autonomy in local decision-making, schools facing accountability pressures do better on international math and science exams (12–14).

What Needs To Be Done?

Critics of NCLB, however, have noted a series of potential problems, including too much focus on basic versus higher-order skills, wide variation in state standards, narrowing of the curriculum, and other distortions in schools. Although the overall importance of each is difficult to pin down with available data and analyses, the underlying ideas suggest useful modifications in the reauthorization of NCLB.

NCLB has each state set learning standards, assessments, and proficiency levels independently, with the federal government determining what actions should be taken when schools fail to make sufficient progress. This division appears to be backward. Under NCLB, states have chosen widely dif-

A federally mandated system of test-based accountability for U.S. education can be made even better.

ferent cutoffs for "proficiency" (15), but in the face of national labor markets where someone from Georgia could well end up working in Arizona, these variations make little sense. History suggests stiff opposition to a national curriculum. As recently seen, however, nothing prevents states from voluntarily joining together to develop standards and assessments (16). The federal government could support and encourage this (17).

On the other hand, the diverse circumstances of schools indicate that centrally defined educational processes are unlikely to be effective (18). The federal government is not well equipped to determine precisely how schools do their jobs. Reforming NCLB could require states to develop their own plans for schools that were failing. Indeed, recognizing the heterogeneity of schools, the U.S. Department of Education has already permitted variation in plans ("differentiated accountability") in nine states (19). As noted above, permitting local autonomy with central testing is a successful strategy consistent with international evidence.

NCLB concentrates on the proportion of students below the state-determined proficiency level in each year. However, accountability should optimally be defined in terms of individual student learning growth, across different learning levels rather than just at the proficiency threshold. This implies that schools should be assessed according to their value added to learning, factoring in such environmental influences as family and neighborhood (20). Fifteen states are already authorized to use growth models for their accountability under NCLB (21). Additionally, this would eliminate incentives to ignore students already above proficiency or too far below to reach proficiency soon.

For testing efficiency, current tests are generally designed to measure most precisely a limited range of skills. An attractive alternative is use of adaptive testing, which can improve measurement in the range of higher-order skills. With adaptive testing (which underlies, for example, the Graduate Record Examination required for entrance to many graduate schools), performance on a set of

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initial screening questions moves the student to the range of test questions most relevant to their demonstrated performance—something easily done with computerized testing. The broadened range of testing would thus encourage expansion of topics and techniques introduced in the classroom rather than a focus on content of a more narrowly constrained test domain. Computerization has two additional advantages. First, it would provide immediate scoring of tests, getting around current delays in test scoring. (Note, however, that accountability testing will not be a substitute for formative assessments in the classroom that are designed to provide feedback to teachers in designing regular instruction plans). Second, having a large test bank would permit providing each student with a random selection of questions, minimizing any chance of cheating. Indeed with a large test bank covering the range of relevant material, it would even be possible to make questions available beforehand, with the notion that “teaching to the test” could actually be considered productive.

Research has found teacher quality to be the most important element of a good school (22), and this underlies the NCLB requirement that all schools have only “highly qualified teachers.” Unfortunately, there are severe measurement problems that make previous interpretations of this requirement hollow at best and harmful at worst. Teacher quality is not captured by characteristics such as master’s degrees, teaching experience, or even certification—things that states typically monitor. Fortunately, TBA produces student achievement data needed to assess the value added of teachers, a more appropriate focus of policy concerns (22).

Conclusions

TBA is a fixture of American education, but it has also become controversial. Clearly, TBA does not do everything, but it is a central part of almost all serious reform efforts. Thus, improving it rather than eliminating it is the only reasonable course.

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EDUCATION

Moving Past No Child Left Behind

Daniel Koretz

No Child Left Behind is a poorly designed program, with serious side effects and little evidence of benefit, in need of fundamental changes.

Weaknesses in the U.S. educational system are clear. U.S. students do not compare well to peers in many other nations in their mastery of mathematics and science (1). Inequities in educational resources and outcomes are glaring. Although policy responses to these problems should include holding educators accountable for student performance, No Child Left Behind (NCLB) is a poorly designed test-based accountability (TBA) system that requires fundamental changes.

Score Inflation

A fundamental problem with TBA systems—one that NCLB fails utterly to address—is score inflation, increases in scores substantially larger than warranted by true gains in students’ learning. Research suggests that inflation, although not ubiquitous, is common and can be very large (2, 3). This phenomenon of corrupted outcome measures is not particular to TBA. It has been seen in many fields where performance-based incentives are imposed (4).

For example, in the early 1990s, Kentucky instituted a TBA system that in many respects

foreshadowed NCLB. During the first 2 years for which teachers were held accountable for scores on the state test, fourth-grade scores on the state’s reading test rose by 0.76 standard deviation (a truly remarkable gain, an order of magnitude larger than historical data would have suggested reasonable). During the same period, the state’s fourth-grade reading scores on a federally managed test [the National Assessment of Educational Progress (NAEP)] did not improve (5), even though the two tests were designed to measure similar material.

Score inflation has several roots, but most important is that achievement tests are necessarily small, somewhat predictable samples of larger domains of achievement. Sample-based testing can work well when educators and students have no strong incentive to focus on the specific sample measured. In TBA systems, however, such incentives are strong (6, 7), and scores sometimes become inflated. Research (8) has indicated that many teachers reallocate instructional time in an effort to focus on tested material (and even on the particular forms in which content appears on the test) at the expense of other content.

Focus on the Proficiency Threshold

These problems must be confronted in the design of any TBA system, but NCLB also

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has specific deficiencies. One is that accountability under NCLB depends almost entirely on one point in the distribution of performance, the so-called “proficient” performance standard. In most instances, educators get credit only for the percentage of students crossing that one line, and reporting focuses on that single statistic. Research is beginning to confirm that such a system can result in undue attention to students thought to be near the cut, at the expense of higher- and lower-scoring students whose improvements do not count for purposes of accountability (9). Even in the absence of such behavior, this form of reporting distorts comparisons of trends between groups that start at different levels of performance (7).

No Strong Evidence of Benefit

Scientifically credible evidence about the effects of NCLB—and of test-based accountability (TBA) more generally—is in short supply. Studies that purport to show net effects on learning are numerous but are as a group too weak to be persuasive. Studies that use scores on accountability tests as an outcome are not credible because of the risk of inflation. Some studies of TBA employ tests less vulnerable to corruption, but they use observational data that make it difficult to distinguish correlation from causation. Some important covariates are poorly measured or missing altogether. These include noneducational variables that directly affect student learning, such as the linguistic environment in the home.

Many of these studies use highly aggregated data—comparisons between states and entire nations—which exacerbates the problem of omitted variables. As the level of aggregation increases, additional variables come into play that may influence achievement. For example, comparisons of educational policies across nations are conflated with cultural differences, which can be a powerful influence on both educational practice and learning. Furthermore, faced with the difficulty of measuring direct influences on achievement (such as family pressure for academic success), many researchers substitute more easily measured proxies, such as socioeconomic status, that are strongly correlated with the unmeasured variables within a single locale. However, the relation between the behavior that directly affects achievement and the proxies may change with aggregation level. Moreover, many of these studies treat educational policies and practices as the cause of differences in achievement, but the causal mechanism may be more complex: Practices may be a response to differences in

achievement, or both may reflect yet other variables.

Simple trends in scores are not conclusive evidence of the impact of NCLB. Instead, we would need to know how trends after the start of the program compare with those that would have appeared without it. However, simple trends are a suggestive starting point, and trends on uninflated measures do not suggest any appreciable impact of NCLB. For example, proponents have noted that fourth-grade mathematics scores on the NAEP have increased since the implementation of NCLB in 2002, but they failed to mention that this improvement was no different from the trend before the bill's enactment. The most recent results of the NAEP showed that fourth-grade mathematics, which had shown rapid score increases for nearly two decades, showed no improvement over the past two years (see fig. S1).

What Fundamental Changes Are Needed

We must look beyond the small number of goals—proficiency in reading, limited aspects of mathematics and science—on which NCLB is focused. Other valued outcomes may get short shrift and may deteriorate if we do not. We need to enumerate the most important goals for education and incorporate as wide a subset as feasible into the accountability system.

Gains in scores must be audited routinely to identify severe inflation and reduce the incentives to engage in inappropriate test preparation. This can be done, for example, by periodic administration of an additional, uncorrupted test, by building audit components into the accountability test, or by evaluating later changes in performance, such as performance in college. The instructional strategies used to raise scores, which will include both real improvements in instruction and undesirable test preparation, must be monitored directly. NCLB creates the same incentives for all agents in the system, to raise scores as quickly as possible. This must change so that those monitoring practice have an incentive to discourage inappropriate test preparation and other gaming. Changes in test design to make inflation more difficult should be encouraged, but they will not suffice.

Human judgment must be reintroduced into the accountability system. Standardized achievement tests are capable of capturing only some of the important goals of education. Human judgment is necessary to capture additional outcomes, evaluate practices,

determine why scores are high or low, and craft interventions when needed. There are many reasons to be leery of adding subjective components to an evaluation system, particularly in a civil service system in which managers lack a profit-based motive to avoid opportunities for abuse.

This problem is more severe in education than in some other fields because of the lack of clear agreement about desirable practices. Given the limits of achievement testing, however, there seems to be no reasonable alternative. Numerous approaches for incorporating judgment have been tried, including inspectorates, quality reviews, peer review, and even parent surveys, but none has yet been adequately evaluated.

Perhaps the most fundamental problem of NCLB and the TBA programs that preceded it is that they have not been based on rigorous research and development (R&D), and they have not been evaluated adequately after implementation. The replacement for NCLB should institute routine and rigorous R&D and evaluation that will provide a scientific basis for better educational accountability systems in the future.

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Supporting Online Material

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MEDICINE

A Comeback for Gene Therapy

Luigi Naldini

Gene therapy has recently had some important successes in treating severe inherited diseases (1–3) after years of skepticism from the scientific community and neglect by the pharmaceutical industry. On page 818 in this issue, Cartier *et al.* (4) report another major advance—the successful first clinical testing of an HIV-derived vector in hematopoietic stem cell (HSC)–based gene therapy. The procedure was used to treat a severe neurodegenerative disease, X-linked adrenoleukodystrophy (ALD), and the results indicate stable expression of a therapeutic gene in a substantial fraction of patients' hematopoietic cells, as well as clinical benefits.

Lentiviral vectors (including HIV-derived) were developed to overcome the inability of gamma-retroviral vectors to infect nondividing cells (5), and have become a widely used gene transfer tool with the potential to extend the reach of gene therapy applications. Several studies have shown that lentiviral vectors transduce HSCs more efficiently than gamma-retroviral vectors (6–11). Cartier *et al.* tested this strategy for treating childhood ALD, a fatal disorder of the central nervous system caused by mutations in *ABCD1*, a peroxisomal transporter gene. The transporter functions in the turnover of myelin (lipid-rich material that insulates neurons) in oligodendrocytes and microglia, and its deficiency leads to demyelination and consequent nervous system dysfunction. Disease progression can be arrested by allogeneic HSC transplantation (cells are from a normal donor), which enables functional myelo-monocytic cells derived from a donor's HSCs to migrate into the recipient's central nervous system and replace diseased microglia cells, thus relieving lipid storage (12).

Gene therapy may provide an alternative treatment if a sufficient number of autologous HSCs

(the stem cell donor is also the patient/recipient) are corrected by gene transfer and successfully engrafted into the patient. But an important question is whether the therapeutic efficacy is comparable to that of transplantation. Also, although the design of a lentivirus vector and its integration preference into the stem cell genome alleviate the risks (11, 13–16) of mutagenesis (and leukemia) that have been observed with gamma-retroviral vectors (2, 17, 18), whether this holds up when lentiviral vectors are used in clinical trials has not been known.

In the study by Cartier *et al.*, two ALD-affected boys were infused with autologous HSCs that were corrected *ex vivo* with an HIV-derived vector expressing *ABCD1* (see the figure). *ABCD1* protein was sta-

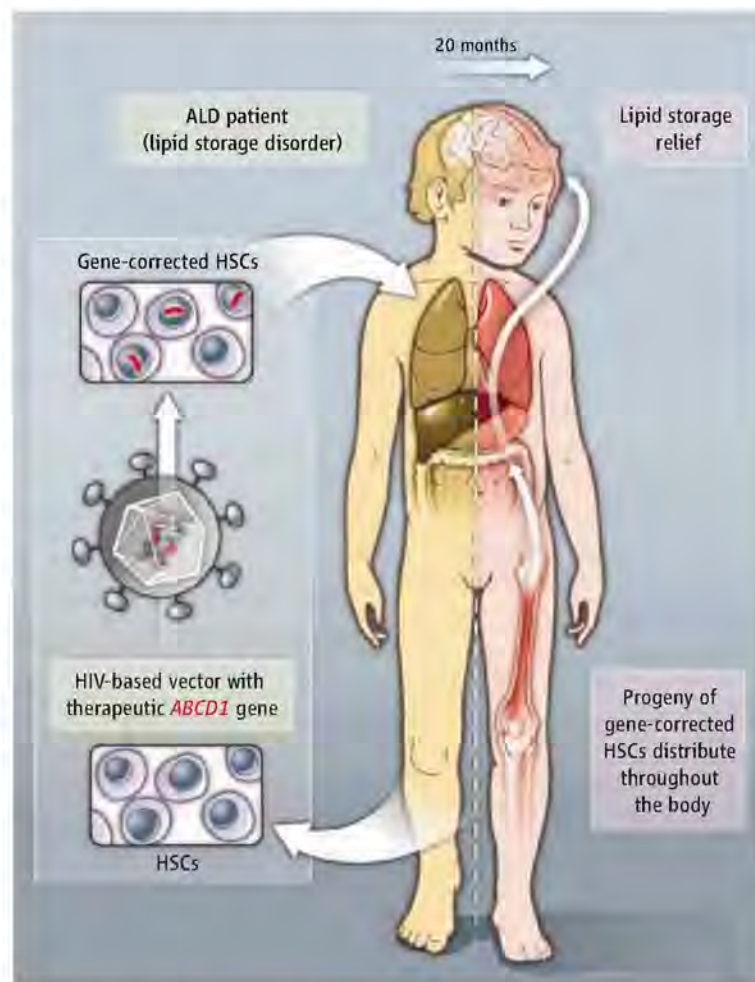
A lentivirus was used as a vector in hemato-poietic stem cells to treat a neurodegenerative disease in a clinical gene therapy trial.

bly expressed in 9 to 14% of granulocytes, monocytes, T and B cells, and bone marrow progenitors in both patients throughout the 24 to 30 months of follow-up, respectively. Cerebral demyelination was arrested 14 to 16 months after engraftment, and neurological and cognitive functions remained stable, an outcome comparable to that of successful HSC transplantation.

Were bona fide HSCs transduced by the lentiviral vector? Mapping integration sites helps to address this question as each site represents a unique genetic marker for tracking the clonal activity of a transduced cell. Up to 4% of all identified vector integration sites were found in both lymphoid and myeloid cells, often at several time points after engraftment. This is close to long-sought evidence for transduction and engraftment of self-renewing, multipotent HSCs.

Was gene transfer into HSCs efficient? As only up to 14% of the cells in each lineage stably expressed *ABCD1*, there is room for improvement. Higher vector titers, which will become available as lentiviral vector manufacturing improves, will likely boost this figure. However, it is difficult to compare earlier gene therapy trials with that of Cartier *et al.*, in which the patient's HSCs were ablated to favor engraftment of the gene-corrected HSCs. Earlier trials based on gamma-retroviral vectors used no or less intense bone marrow conditioning and exploited the growth advantage of gene-corrected cells to favor their engraftment and expansion *in vivo* (1, 2, 17).

Long-term engraftment of hematopoietic cells transduced by gamma-retroviral vectors is often characterized by the appearance of clonal cells bearing vector integration in genes involved in growth control and/or oncogenesis. This



Promising treatment. Progeny of HSCs that were engineered to carry the correct version of a gene (through the integration of a lentiviral vector) distribute throughout the body. Cartier *et al.* show that some cells replaced diseased microglia in the brain and relieved lipid storage in patients suffering from ALD.

may indicate positive selection of clones with increased growth potential caused by vector insertion (2, 17, 18). Is lentiviral vector integration more neutral? Cartier *et al.* report a reassuring picture of highly polyclonal hematopoietic cells transduced with the *ABCD1* gene, which is maintained throughout the follow-up time without evidence for sustained expansion of individual clones or enrichment of common integration sites. But when the authors compared the distribution of integration sites in cells before infusion and after engraftment, they observed an enrichment of integration sites at some gene classes after engraftment. This may suggest that integration was not completely neutral. It may also reflect differences in integration preference between the short-lived progenitors, which constitute most of the cells infused into the patients, and the rare HSCs whose progeny engraft the patients long-term. Longer follow-up and additional testing in this and other diseases will better establish the safety features of lentiviral vectors and how they can be influenced by conditions specific to each study design.

If most lentiviral vector integration is neutral to cell behavior, the tracking of integra-

tion site distribution in the different cell lineages reported by Cartier *et al.* may be a first glimpse of live hematopoiesis in humans at the clonal level. The authors used a combination of approaches to maximize the coverage of integration sites in each sample and alleviate the retrieval biases imposed by the DNA restriction and amplification steps of the procedure. This technological rigor will likely become a gold standard for future HSC-based gene therapy trials.

Gene therapy of ALD in the study of Cartier *et al.* provided a benefit similar to that of allogeneic HSC transplantation, despite a relatively low level of gene correction. This unexpected finding indicates that enhanced efficacy in relieving lipid storage may be attained with cells that overexpress the therapeutic gene as compared to normal donor cells. It also suggests that microglia cells might be replaced by infused short-lived progenitors that contain a higher proportion of gene-corrected cells than HSCs. These scenarios might eventually position HSC-based gene therapy as a preferable treatment option for ALD, as it abrogates the morbidity associated with the allogeneic source of HSCs in conventional transplantation. Furthermore,

improved HSC transduction protocols may overcome the need for bone marrow conditioning. Although many questions remain to be fully settled, this study clearly supports further testing of HSC-based gene therapy in ALD and other diseases and represents a long-sought rewarding achievement in the field of gene therapy.

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ECOLOGY

Biodiversity and Climate Change

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Over the past decade, several models have been developed to predict the impact of climate change on biodiversity. Results from these models have suggested some alarming consequences of climate change for biodiversity, predicting, for example, that in the next century many plants and animals will go extinct (1) and there could be a large-scale dieback of tropical rainforests (2). However, caution may be required in interpreting results from these models, not least because their coarse spatial scales fail to capture topography or “microclimatic buffering” and they often do not consider the full acclimation capacity of plants and animals (3). Several recent studies indicate that taking these factors into consideration can seriously alter the model predictions (4–7).

In one study, Randin *et al.* assessed the influence of spatial scale on the accuracy of

bioclimatic model predictions of habitat losses for alpine plant species in the Swiss Alps (4). A coarse European-scale model (with 16 km by 16 km grid cells) predicted a loss of all suitable habitats during the 21st century, whereas a model run using local-scale data (25 m by 25 m grid cells) predicted persistence of suitable habitats for up to 100% of plant species. The authors attributed these differences to the failure of the coarser spatial-scale model to capture local topographic diversity, as well as the complexity of spatial patterns in climate driven by topography.

Luoto and Heikkinen reached a similar conclusion in their study of the predictive accuracy of bioclimatic envelope models (which model the relation between current climate variables and present-day species distributions) on the future distribution of 100 European butterfly species (5). A model that included climate and topographical heterogeneity (such as elevational range) predicted only half of the species losses in mountainous areas for the period from 2051 to 2080 in comparison to a climate-

Efforts to elucidate the effect of climate change on biodiversity with detailed data sets and refined models reach novel conclusions.

only model. In contrast, the number of species predicted to disappear from flatlands doubled in the climate-topography model relative to the climate-only model. The two studies suggest that habitat heterogeneity resulting from topographic diversity may be essential for persistence of biota in a future changing climate.

Highly contrasting predictions have also been obtained when bioclimatic models of tropical biomes included the physiological effects of elevated atmospheric CO₂ concentrations and temperature on trees (6). Many studies have indicated that increased atmospheric CO₂ affects photosynthesis rates and enhances net primary productivity—more so in tropical than in temperate regions—yet previous climate-vegetation simulations did not take this into account.

To address these issues, Lapola *et al.* (6) developed a new potential-vegetation model for tropical South America that includes CO₂ fertilization effects. They then drove this model with different climate scenarios for the end of the 21st century from 14 coupled

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ocean-atmosphere global climate models of the Intergovernmental Panel on Climate Change (IPCC) Fourth Assessment report (8). The results indicate that when the CO₂ fertilization effects are considered, they overwhelm the impacts arising from temperature; rather than the large-scale die-back predicted previously (2), tropical rainforest biomes remain the same or substituted by wetter and more productive biomes. However, for 2 of the 14 models, this result was dependent on the dry season not extending beyond 4 months; if it does, then the tropical biome becomes savanna (6).

These studies highlight the level of complexity that we are faced with in trying to model and predict the possible consequences of future climate change on biodiversity (9). They suggest that we should expect to see species turnover, migrations, and novel communities, but not necessarily the levels of extinction previously predicted. For example, Hole *et al.* recently studied model-projected shifts in the distribution of sub-Saharan Africa's breeding bird fauna. They found that in the Important Bird Area protected network, species turnover is likely to be substantial and regionally variable, but persistence of suitable climate space across the network as a whole is remarkably high, with 88 to 92% of species retaining suitable climate space (7).

Another complexity, however, is the impact of climate change on already highly altered fragmented landscapes outside of protected areas. Over 75% of the Earth's terrestrial biomes now show evidence of alteration as a result of human residence and land use (10). Yet, recent case studies suggest that even in a highly fragmented landscape, all is not lost for biodiversity.

It has long been assumed that in a fragmented landscape, the fragment size and its isolation are important factors in determining species persistence; the smaller and more isolated the fragment, the lower its occupancy. Yet few worldwide studies have attempted to quantify this relation. Prugh *et al.* (11) compiled and analyzed raw data from previous research on the occurrence of 785 animal species in >12,000 discrete habitat fragments on six continents. In many cases, fragment size and isolation were poor predictors of occupancy. The quality of the matrix surrounding the fragment had a greater influence on persistence: When the matrix provided conditions suitable to live and reproduce, fragment size and isolation were less important and species were able to persist.

This ability of species to persist in what would appear to be a highly undesirable and



Looking beyond reserves. This photo of the boundary between the Mfungabusi Forest, Zimbabwe, and surrounding farmland highlights the contrast between protected and nonprotected landscapes.

fragmented landscape has also been recently demonstrated in West Africa. In a census on the presence of 972 forest butterflies over the past 16 years, Larsen found that despite an 87% reduction in forest cover, 97% of all species ever recorded in the area are still present (12). For reasons that are not entirely clear, these butterfly species appear to be able to survive in the remaining primary and secondary forest fragments and disturbed lands in the West African rainforest. However, presence or absence does not take into account lag effects of declining populations; a more worrying interpretation is therefore that the full effects of fragmentation will only be seen in future years.

Predicting the fate of biodiversity in response to climate change combined with habitat fragmentation is a serious undertaking fraught with caveats and complexities. The recent studies discussed here attempt to quantify some of the uncertainty in these predictions. They use larger, more detailed data sets and more-refined models than previously available, thus avoiding the problems often encountered in trying to scale up results from small local-scale studies.

The results also highlight a serious issue for future conservationists: the urgent need to develop a research agenda for regions outside of protected reserves in human-modified landscapes (see the figure) (13). Although every measure should be put in place to reduce further

fragmentation of reserves, we must determine what represents a "good" intervening matrix in these human-modified landscapes (11–14). Furthermore, with the combination of climate change and habitat destruction, novel ecosystems are going to become increasingly common. Their conservation will require a whole new definition of what is "natural" (15).

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PALEONTOLOGY

Evolution of Animal Pollination

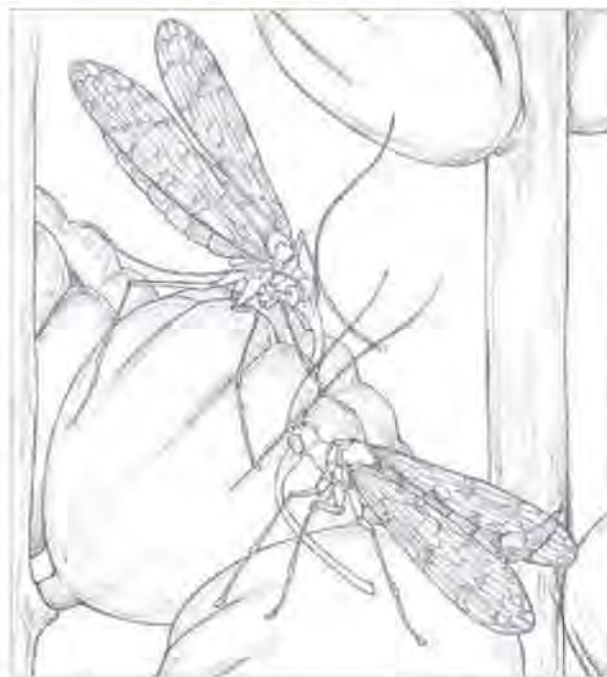
Jeff Ollerton and Emma Coulthard

The evolution of animal pollination in flowering plants (angiosperms) and the resulting coevolution and diversification of both angiosperms and major pollinator groups during the late Cretaceous (99.6 to 65.5 million years ago) is one of the classic stories of evolutionary biology (1). On page 840 of this issue, however, Ren *et al.* (2) challenge aspects of this story and hint at a much more complex ecological scenario for the evolution of plant-pollinator relationships.

An important feature of the traditional story is that the non-angiosperm seed plants living during the Mesozoic (251 to 65.5 million years ago) were mainly wind-pollinated. Although the fossil record of these plants shows evidence of possible animal pollination as early as the late Carboniferous (320 to 300 million years ago) (3), this evidence is open to interpretations of the size of pollen grains (apparently too large to be wind-dispersed), the possibly attractive function of reproductive organs, and patterns of damage by insects. The assumption is that although animal pollination may predate the evolution of the flowering plants, it was rare and unspecialized relative to what was to follow in the late Cretaceous (4).

Ren *et al.* now marshal evidence from an impressive range of sources—from comparative morphology of fossil insect mouthparts to elemental analysis of the fossils and their surrounding matrix—to propose that a previously overlooked group of Mesozoic scorpionflies was able to feed on a nectarlike fluid (5) produced by a group of now-extinct non-angiosperm seed plants. The authors suggest that the scorpionflies in turn pollinated the plants. This may be the earliest known example of coevolution between plants and pollinators. The evidence that Ren *et al.* present is compelling, and if they are correct, it will change our understanding of the early ecology and evolution of pollination by insects.

As Darwin (6) famously recognized when he speculated about the coevolution of flower and tongue length between the Madagascan orchid *Angraecum sesquipedale* and its (then unknown) moth pollinator, flowering plants have often evolved tubular structures that



Ancient legacy. Mesozoic scorpionflies (Mecoptera) probe the female reproductive organs of *Leptostrobus cancer*, a member of the extinct order Czekanowskiales. Modern scorpionflies are much less diverse than their Mesozoic antecedents. Although they only rarely visit flowers, this habit may be overlooked in modern species and could be a legacy of their distant ancestors.

hold nectar or protect reproductive organs. The plants can thus discriminate between flower visitors, enabling them to specialize on pollinators with appropriately sized mouthparts (7, 8). This match between mouthparts and flower depth (9) facilitates more accurate pollen placement, meaning that less pollen is wasted, and prevents nonpollinating animals from robbing nectar. It may be a major factor driving the diversification of some angiosperm genera (8) and structuring the patterns of interaction with pollinators in plant communities (10), but until Ren *et al.*'s study, it was considered unimportant in non-angiosperm pollination.

The 11 scorpionfly species described by Ren *et al.* have mouthparts that are both relatively long and consistent with fluid feeding by sucking. The species represent three different families, pointing to repeated convergent evolution of this feeding strategy, which in turn suggests that substantial quantities of nectarlike fluid (5) were available to these taxa. Ren *et al.* believe that the source was a group of non-angiosperm seed plants belonging to diverse, and mostly extinct, lineages.

Animals pollinated specialized seed plants even before flowering plants evolved.

All these species have reproductive organs that are poorly adapted to wind pollination (the previously presumed mode of reproduction for these taxa). Wind pollination requires that pollen-receptive areas are easily accessible to windborne pollen, which is clearly not the case for these plants. Pollen transfer by insects thus seems most likely, and the scorpionflies are the best candidates so far identified.

Some will find these claims controversial, particularly as a key piece of evidence is missing: The authors failed to find any pollen associated with these fossils. This is especially surprising for the amber-encased insects, where pollen preservation would be expected (11). Absence of evidence is not, however, evidence of absence, and further fossils may provide this information.

Biotic pollination was the dominant angiosperm pollination strategy by the late Cretaceous (12). Ren *et al.*'s research tests old notions that angiosperms evolved in a predominantly wind-pollinated world. Furthermore, it challenges us to rethink assumptions that early pollinators were short-tongued generalists that could only exploit open flowers with easily accessible nectar. Living representatives of the earliest diverging flowering plants have a diverse range of pollination systems (13), and many are far from generalized in their interactions with pollinators. The presence of long-tongued pollinating scorpionflies both before and at the same time as the first angiosperms allows us to imagine that flowering plants evolved deep flowers early in their radiation, coexisting with open, generalist flowers and gymnosperms as part of a specialist-generalist spectrum similar to modern plant communities (14).

Can modern assemblages of plants and pollinators be viewed as analogous to their Mesozoic counterparts, at least in terms of functionality, if not phylogenetic identity? Other Mesozoic insects have been suggested to be fluid-feeding pollinators (15, 16), and if this is so, then the scorpionflies described by

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Ren *et al.* were probably part of a larger fauna of insects visiting plant reproductive structures. The Mesozoic Era was biotically richer and more complex than previously realized. Studies such as that by Ren *et al.* provide a tantalizing glimpse of lost worlds of interactions between partners that are now extinct or considerably less diverse.

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CHEMISTRY

Capturing the Complexities of Molecule-Surface Interactions

Eckart Hasselbrink

The simplest picture of a chemical reaction is that two molecules approach, climb a potential energy barrier as bonds get pulled apart in the transition state, and then separate, forming the new products. Molecules move in three dimensions and have internal motions such as vibrations, so a quantitative model requires molecules to move over a potential energy surface (1). Two reports in this issue address the added complexities that result when one of the reactants is a metal surface (see the figure). On page 829, Shenvi *et al.* (2) describe a method for the quantitative evaluation of one of the open questions in modeling these reactions: How is energy dissipated as the molecule approaches the metal surface? On page 832, Diaz *et al.* (3) present a pragmatic fix for the problem of calculating the potential energy surface that describes how molecular hydrogen (H_2) reacts with an atomically flat copper surface. Their approach allows almost every aspect of the experimental findings for this system to be reproduced.

For reactions between small molecules in the gas phase, potential energy surfaces calculated from first principles (without using inputs from experiments) have become quite successful in predicting product formation (4). However, for the more complex case of molecules interacting with a metal surface—which is of interest for understanding reactions in industrial catalysts—getting the calculations to agree with experiment is much more challenging, because the system to consider is extended and because energy dissipation to electronic excitations may become important (5).

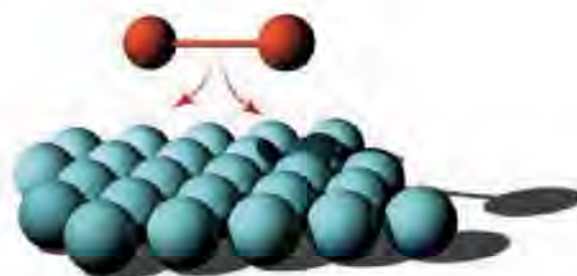
When molecules meet surfaces. The interaction of a molecule with a metal surface provides a challenge for the most sophisticated molecular structure calculations. Theory is rapidly moving toward a quantitative account of how molecules scatter off surfaces, as discussed by Shenvi *et al.*, or react and form bound surface species, as discussed by Diaz *et al.*

Modeling a chemical reaction is typically a two-step process. First, the interaction energy of the reactants is calculated for various geometric arrangements and then used to create the potential energy surface. The latter is then the basis for the calculation of the dynamics—that is, how the reactants will move and exchange energy until the products are formed. These analyses can be used to calculate reaction probabilities for real systems, where the molecules have a distribution of energies.

This two-step procedure rests on the thesis work of Robert Oppenheimer: the so-called Born-Oppenheimer approximation (BOA), which allows calculations of electronic energy independent of the motion (such as bond vibrations) of the much heavier nuclei (6). This approximation has been very successful for predicting molecular structures and reaction dynamics between molecules. However, the interaction with a metal surface is a particularly problematic case for applying the BOA because metals have a continuous electronic excitation spectrum, not discrete energy levels like single atoms and molecules.

It has been rigorously shown that the interaction of a molecule with a metal surface must dissipate energy into substrate electronic excitations; processes that dissipate energy in this way are called nonadiabatic (7–9). However, it is still not established how to predict the mag-

Two large computational studies describe how theory can better account for the way in which molecules scatter from or react with metal surfaces.



nitude of this energy, and the error introduced by the BOA, for a particular reaction system. The steadily increasing number of experimental studies that have reported evidence of non-adiabatic behavior emphasizes the need to understand the error created by the BOA [see (10) and references therein].

Shenvi *et al.* present a method that allows a quantitative account of nonadiabaticity and apply it to the particular case of a nitric oxide (NO) molecule scattering off a gold surface. They use data from a sophisticated experiment as a benchmark for their theoretical study. Wodtke and co-workers prepared NO molecules in a highly vibrationally excited state by means of laser techniques and observed that it loses vibrational energy several quanta at a time in the collision (11). Their experimental data are only consistent with a non-adiabatic coupling mechanism. An adiabatic model for this interaction would predict that the molecules scatter from a metal surface with the vibrational excitation intact, because the vibrational frequency is not in resonance with the vibrations of the surface atoms or the molecule-surface bond.

The vibrational motion of the NO molecule is connected with an oscillating shift in electronic energy for adding one more electron to the NO molecule. The vibrational motion is hence connected with an oscillat-

ing flow of charge back and forth between the NO molecule and the valence electrons in the metal. Thus, the coupling to substrate electronic excitations becomes strong. Further credence for this interpretation is given by other experiments (12) showing that vibrational energy can cause ejection of low-energy surface electrons.

Shenvi *et al.* represent the continuum of electronic states by considering the NO molecule in its neutral and ionic states coupled to 40 discrete substrate states, each one having its own potential energy surface. They allow the molecule to jump randomly along its way between these discrete states. This demanding calculation reproduces the relaxation of the molecule seen by the experimentalists.

Further analysis of these results suggests that the molecule is steered during the scattering event into an orientation favorable for electronic coupling. Shenvi *et al.* also make predictions that could be tested experimentally, but more important, their results corroborate the strongly nonadiabatic character of the scattering process. One concern is that these effects might be specific to molecules like NO that have an odd number of electrons. However, similar effects in more typical closed-shell molecules may be more difficult to identify unambiguously.

In contrast, nonadiabatic effects are a minor concern in the dissociative adsorption

of H₂ (the formation of two bound H atoms) on the flat copper surface [the close-packed (111) face]. To describe the interaction of the molecule with the surface, Diaz *et al.* must use density functional theory (DFT), but at present, no functionals—which are at the heart of DFT—have been developed that achieve for gas-surface systems a good description of the stable bonding situation as well as the transition state separating reactants and products.

Given this difficulty, the authors made the brave assumption that the real potential energy surface is straddled by the ones obtained from calculations based on two different and widely used functionals. They merged the two results by weighting them with an adjustable parameter. As Diaz *et al.* show, this potential energy surface results in excellent agreement with extensive experimental results that have been obtained for this particular system at the level of individual quantum states. They emphasize that it is crucial to include in the calculation all six nuclear degrees of freedom of the scattering molecule. As a larger number of degrees of freedom comes at high computational cost, they treat the surface atoms as frozen in space.

In some sense, the authors of the two reports work from opposing ends of the general problem of describing molecule-surface interactions. Shenvi *et al.* try to improve ab initio methods by nailing down the error

induced by one suspicious approximation. Diaz *et al.* take a pragmatic approach and show that, in principle, a potential energy surface exists that allows calculations to reproduce experiments quantitatively applying adiabatic dynamics and that serves as a target for future, more exact calculations. It remains to be seen to what extent their approach is generalizable, or if canceling errors have led to fortuitous results in this system. In any case, both reports demonstrate that gas-surface dynamics provide a sensitive test for the theories used to calculate molecular structures.

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ENVIRONMENTAL SCIENCE

Peatland Response to Global Change

Nancy B. Dise

Meter for meter, peatlands store more carbon than any other terrestrial ecosystem. Covering only about 3% of Earth's land area, they hold the equivalent of half of the carbon that is in the atmosphere as CO₂ (1, 2). Waterlogged conditions slow decomposition, and slow rates of subsurface flow allow the partly decayed organic matter to accumulate in place. But the same processes of anaerobic decomposition that allow carbon to accumulate also produce the strong greenhouse gas methane (CH₄). Over the time span of centuries, peatlands exert a

net cooling effect on the global radiation balance, because the effect of removing long-lived atmospheric CO₂ ultimately surpasses that of releasing short-lived CH₄ (3). However, should peatlands begin to degrade on a large scale, this stored carbon could be released, reducing—or even reversing—their climate cooling effect. How will the carbon balance of peatlands change over coming centuries?

The clearest threat to peatlands today is direct damage by humans: agricultural conversion or drainage (for example for rice, palm oil, or forests) and mining (for horticulture and fuel). Far less obvious, but potentially as damaging, however, are long-term environmental changes such as global warming. Most peatlands are located in the boreal and subarctic Northern Hemisphere, where

Peatlands can buffer the impact of external perturbations, but can also rapidly shift to a new ecosystem type, with large gains or losses of stored carbon.

the climate is warming faster than anywhere else on Earth (2). Peatlands are also affected by numerous other environmental factors that are likely to change in the future, including precipitation amount and frequency, atmospheric deposition of reactive nitrogen and sulfur, atmospheric CO₂ levels, extreme weather, and fire. These drivers interact in complex ways, and predicting their net effect will not be possible by simply attempting to combine individual impacts.

Research from a variety of areas and approaches is converging upon the concept of peatlands as complex adaptive systems: self-regulating to some degree, but capable of rapid change and reorganization in response to internal developmental changes or to external forcing (4). It has long been known,

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for instance, that the surface of a peatland can rise and fall, sometimes dramatically, in response to rainfall or mild drought, while maintaining a fairly constant water level relative to the surface. This “Mooratmung” (“bog-breathing”) is related to the sponge-like nature of *Sphagnum*, which can adsorb water and trap gases. Recent studies have shown that the carbon balance of peatlands can in turn be surprisingly resilient to perturbations, even fairly severe ones. For example, subjecting peat cores (5) or a peatland field site (6) to a water table drawdown similar to a prolonged drought initially led to a respiration-driven loss of soil carbon. But both carbon loss and subsidence (6) lowered the peat

mate has become wet enough for low microsites to flood, creating a ridge- and hollow-patterned peatland with a much-diminished capacity to sequester carbon

The responses to global warming of peatlands and tundra in the vast boreal/subarctic region underlain by permafrost may also follow the pattern of a complex adaptive system. Analysis of peat cores in boreal Canada covering a 150-year period showed that thawing of raised peat underlain by discontinuous permafrost caused the surface to collapse to near the level of the water table (9). This collapse induced a rapid vegetation change from species characteristic of dry, cold climates to *Sphagnum* species characteristic of wet

during the transition. However, even if some net carbon accumulation returns, the gains are short-lived: The key peatland quality of slowly removing and storing carbon for hundreds or thousands of years is lost.

Considering peatlands as complex adaptive systems characterized by quasistable equilibrium states—resilient to change at some level of perturbation but shifting to new states at higher levels of disturbance—provides a meaningful framework for understanding and modeling their response to environmental change. Ignoring the strong feedbacks inherent in peatlands may lead to substantial under- or overestimates of their response to global change. The challenge is to forecast both the



Shifting states. (Left) Hummock-hollow pattern at Rygmossen, a small raised bog near Uppsala, Sweden. (Right) “Ladder” system of ridges and pools, Inverewe



Bogs, Scotland. Persistent environmental change, such as a long-term increase in climate wetness, can trigger a shift from one such peatland type to another.

surface, decreasing its height above the water table, and effectively shifted the system back toward its starting state. Conversely, a rising water table stimulated growth of *Sphagnum* and other vegetation, which increased carbon accumulation, raised the surface of the peat and, in effect, lowered the local water table (5). Thus, an environmental perturbation may trigger an initial gain or loss of carbon, but recovery in the direction of the initial state can moderate the impact.

Subject to a stronger or more persistent environmental change, a peatland may shift from one state to another (see the figure). Analysis of cores from a bog in Sweden showed a succession of three different *Sphagnum* assemblages, each characteristic of progressively wetter conditions, in response to an increase in precipitation over 5000 years (7, 8). Transitions were sudden (years to decades) and accompanied by a major increase in the rate of carbon accumulation. Between transitions, carbon accumulation rates slowly declined, in part [as in (5)] because growth of *Sphagnum* raised the surface of the peat in relation to the water table. However, over the past 1000 years, carbon accumulation rates have declined sharply. It appears that the cli-

depressions, increasing both carbon accumulation and CH_4 emission. Both rates then gradually declined as the ecosystem developed into a continental bog. Given that these bogs characteristically emit low levels of CH_4 and steadily sequester carbon, the authors concluded that in the long-term, conversion from permafrost peatland to lawn and finally to bog in western Canada has a climate cooling effect.

However, warming and drying without surface collapse can result in substantial carbon loss, as shown in the initial years of a long-term field study in Alaskan Arctic tundra (10). Overall, whether carbon is gained or lost depends on whether the transition is toward or away from the optimal conditions for carbon accumulation for that ecosystem, and this is mainly determined by the hydrologic response.

Long-term global changes—particularly warming, drought, and elevated nitrogen deposition—are likely to ultimately induce shifts in some existing peat-forming areas to new ecosystems such as grassland or shrubland (10, 11), and the increase in biomass from vascular plants could in part compensate for carbon losses from soil oxidation

future environmental conditions that peatlands will experience and the internal feedbacks and state changes that may be triggered by these conditions. To meet this challenge it is vital to continue and expand long-term monitoring networks to characterize the present, paleo-environment research to reconstruct the past, and manipulation experiments in the field and laboratory to build our understanding of these unique and valuable ecosystems.

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Einstein's Theory of Gravity and the Problem of Missing Mass

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The observed matter in the universe accounts for just 5% of the observed gravity. A possible explanation is that Newton's and Einstein's theories of gravity fail where gravity is either weak or enhanced. The modified theory of Newtonian dynamics (MOND) reproduces, without dark matter, spiral-galaxy orbital motions and the relation between luminosity and rotation in galaxies, although not in clusters. Recent extensions of Einstein's theory are theoretically more complete. They inevitably include dark fields that seed structure growth, and they may explain recent weak lensing data. However, the presence of dark fields reduces calculability and comes at the expense of the original MOND premise, that the matter we see is the sole source of gravity. Observational tests of the relic radiation, weak lensing, and the growth of structure may distinguish modified gravity from dark matter.

The problem of missing mass has been with us for more than 70 years: Given the amount of directly observable matter, general relativity (GR, Einstein's theory of gravity) produces too little gravity to account for a host of observations. On scales of one to tens of kiloparsecs, the observed random or coherent velocities of stars and gas are much greater than the escape velocity in the self-gravity of those same stars, gas, and dust. The same is true for galaxy clusters on much larger scales. Gravitational potentials around galaxy clusters are deeper than what would be produced by the observed matter are also needed to explain observed gravitational lensing; that is, the deformation of light bundles from background galaxies.

Evidence also exists for anomalously strong gravity on the largest observable scale: out to the cosmological horizon. In a universe that contained only ordinary matter (often called baryonic matter, encompassing protons and neutrons, which make up over 99.9% of the mass of ordinary matter), the growth of structures such as galaxies and clusters of galaxies would be suppressed. During recombination, when that universe was approximately 400,000 years old, the seeds for galaxies and clusters would be entirely erased by dissipation (known as Silk damping), and no structure would form on scales up to many tens of megaparsecs.

The now-standard solution of these dynamical mysteries is that an unobserved form of mass, which exceeds the observed mass of both galaxies and clusters, provides the gravity that

prevents them from flying apart, increases lensing, and prevents Silk damping. The missing mass neither shines nor absorbs nor scatters light enough to be directly detected by our telescopes. It should be close to pressureless and must be nonrelativistic well before recombination. It is therefore known as cold dark matter (CDM). A number of candidate particles have been pro-

posed that have these properties, and their behavior in the universe has been studied in exquisite detail. An altogether different approach can be taken if one notes that the evidence for missing mass arises because of a mismatch between the gravitational field one would predict from the observed mass distribution in the universe and the observed gravitational field. The observed discrepancies arise when the effective gravitational acceleration is around or below $a_0 \approx 10^{-8} \text{ cm s}^{-2}$; that is, in a regime of very weak gravitational field. Perhaps the Newtonian theory of gravity and GR break down in this regime. In this Review, we provide an updated assessment of this theory.

Modifying Newtonian Gravity

The possibility that Newtonian gravity and GR do not accurately describe very weak gravitational fields was proposed more than 25 years ago. Milgrom suggested that Newton's second law, $F = m\vec{a}$ (where F is the gravitational force applied to a unit of mass m to produce an acceleration $\vec{a}$), is modified when gravity is weak, to $\vec{F} = m(|\vec{a}|/a_0)\vec{a}$ (1). This proposal has been named modified Newtonian dynamics (MOND). More-modern versions of MOND cast it instead as a modified theory of gravity, altering the Newton-Poisson equation that relates the gravi-

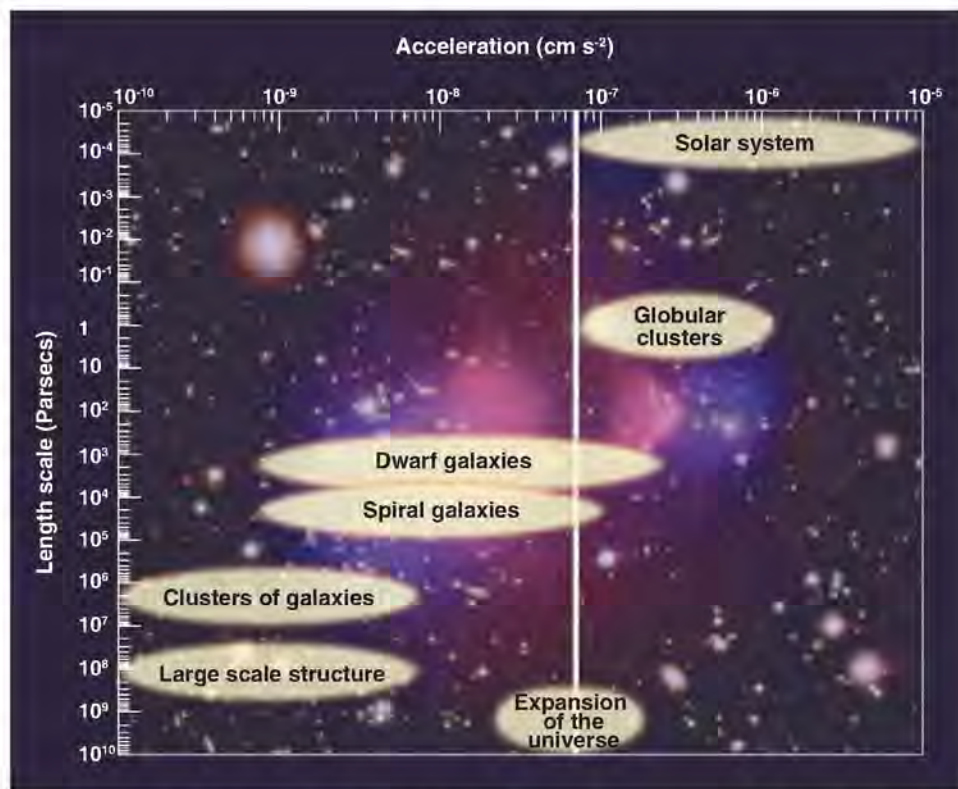


Fig. 1. Evidence for dark matter or for deviations from GR tend to appear in systems in which the acceleration scale is weak (below the solid horizontal line) at about $10^{-8} \text{ cm s}^{-2}$. There is strong evidence for either of the above in dwarf galaxies, spiral galaxies, clusters of galaxies, the large-scale structure of the universe, and in the expansion of the universe itself. [Source: X-ray: NASA/CXC/CfA/M. Markevitch *et al.*; Optical: NASA/STScI; Magellan/U. Arizona/D. Clowe *et al.*; Lensing Map: NASA/STScI; ESO WFI; Magellan/U. Arizona/D. Clowe *et al.*]

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tational force to the distribution of mass density ρ that is responsible for it (2).

MOND has a number of appealing features. It independently explains the empirical Tully-Fisher relation between the luminosity L of a spiral galaxy and its asymptotic rotational velocity v : $L \propto v^4$. Given the ratio of the baryonic mass of these spirals to their luminosity (the mass-to-light ratio), this is equivalent to $(a_0 G)M = v^4$, where G is the gravitational constant and M is the baryonic mass of the galaxy, exactly what would be predicted by MOND. A systematic study of a wide range of spiral galaxies pins the acceleration scale to be unique: $a_0 \approx 1.2 \times 10^{-8} \text{ cm s}^{-2}$. Furthermore, the detailed features of the rotational velocity as a function of radius are predicted by the baryonic mass distribution (3). MOND has also been used to predict the analog of the Tully-Fisher relation for elliptical galaxies (the Faber-Jackson relation between baryonic mass and velocity dispersion), the existence of galaxies with low surface brightness, and an upper limit on the mean surface brightness of spiral galaxies (known as Freeman's law) (4).

Attempts to resolve the mass discrepancy on the scale of clusters of galaxies have been more problematic. One is obliged either to use a value of a_0 that is different from the one used for galaxies, or to assume the existence of a small amount of dark matter. If one of the three types of neutrino (electron, muon, or tau) has a mass of a few electrons volts, it would be an ideal candidate for cluster dark matter in MOND (5) (Fig. 1).

An added complication is that gravitating systems cannot be studied in isolation, and the external gravitational field can play a role in the internal dynamics of disparate objects such as star clusters, molecular clouds, and galaxies. MOND does not satisfy Birkhoff's theorem (the analog in gravity of Gauss' law in electromagnetism) for real masses (6–8), and this means that the acceleration of any real probe—a star or a cloud of gas—even if it is located in a spherically symmetric system, depends not just on the mass that is interior to the probe but on the mass that is exterior as well.

MOND was developed as a phenomenological description of spherically (or at least axially) symmetric, nonrelativistic, low-acceleration systems. Until a fully dynamical relativistic theory can be constructed, MOND itself cannot reliably predict, among other things, anomalous accelerations in galaxy clusters, the effects of gravitational lensing of light, the expansion of the universe, or the growth of structure.

Relativistic Theories of Modified Gravity

Despite MOND's successes and failures, for it to be seriously judged as a candidate explanation of anything, it must be embedded in a modification of Einstein's GR theory. Einstein cast gravity as a geometric theory of spacetime (the combination of space and time). The properties of spacetime are encoded in a 4×4 symmetric matrix with 10 free components, which is called the metric g , which is itself a function of space and time. From

this metric, one can construct various geometric quantities such as the overall curvature of spacetime R , known as the Ricci scalar, the Ricci tensor $\mathbf{R}$, and the Riemann tensor $\mathcal{R}$.

Einstein proposed that the energy content of the universe would source these various quantities, curving spacetime according to a fixed set of rules called the Einstein field equations. The different components of the universe would, in turn, respond to the curvature of spacetime: In the absence of nongravitational forces, they would follow geodesic paths that could be derived from the metric. Einstein's theory must be tampered with to incorporate MOND. There are two possible ways of modifying it. One way is to change how curvature responds to the pres-

there is an extensive program to explore the theoretical and phenomenological consequences.

A complementary approach is to postulate that light and matter respond to the geometry of space and time differently than predicted by Einstein. The simplest way to implement this is to distinguish the geometric metric, which responds dynamically to the contents of the universe, from the geodesic metric, which dictates (in the absence of other, nongravitational, forces) how those contents propagate through spacetime. The simplest such theory relates the two metrics by a location-dependent change of scale, known as a conformal transformation, and endows the scalar field describing this transformation with dynamics of its own. This theory has been extensively studied

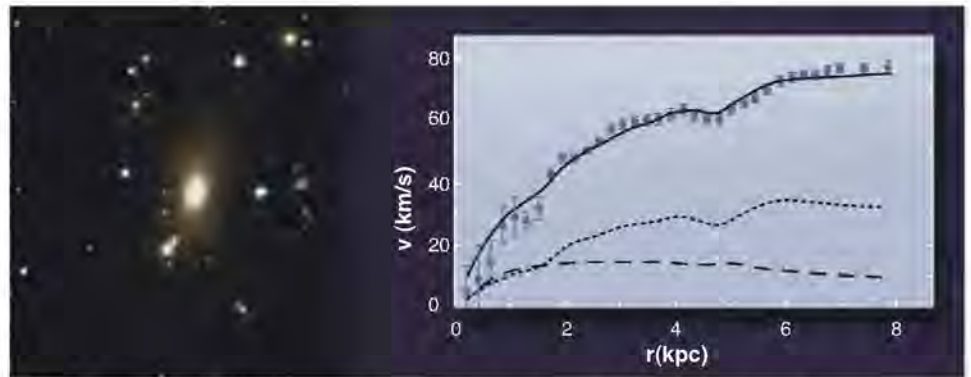


Fig. 2. The MOND rotation curve of the galaxy NGC1650 (solid line) reproduces observed features with surprising fidelity given just one free parameter: the mass-to-light ratio $M/L = 0.43$. Also shown is the Newtonian rotation curve that would result from just the gas (dotted) or just the stars (dashed). The quality of the rotation curve fit from MOND is generic. [Figure courtesy of Stacy McGaugh; originally published in (42); image from www.sky-map.org]

ence of matter. The rules that Einstein posited for deriving gravity start with the simplest of all the quantities that encode curvature: the Ricci scalar. A first step away from Einstein is to bring in other functions of the metric such as the Ricci and Riemann tensors, as well as more-complicated functions of the Ricci scalar. Indeed, because of the effects of quantum mechanics on the spacetime metric, one expects the full theory of gravity to be a more complex combination of various geometric quantities (9). As a result, one inevitably introduces new gravitational degrees of freedom.

Some of these modifications have consequences at short distance scales and result in small (although potentially measurable) corrections to standard physics, which are insufficient to reproduce galactic rotation curves (Fig. 2). Others result in modifications to cosmology, but not in MOND-like behavior on galactic scales. Nevertheless, a few proposals have been advanced for modifications of gravity that can play a role on galactic and supragalactic scales. In (10), the Ricci tensor is replaced by the Weyl curvature, and a scalar field is introduced to play the role of a variable Newton's constant. In (11), a logarithm of the Ricci curvature is considered as the fundamental action. For these cases and others,

in many contexts and is highly constrained (12). Whereas such a scalar field can affect the dynamics of massive bodies, it does not modify the propagation of light rays and therefore will not play a role in phenomena such as gravitational lensing.

More general transformations involve introducing not only a change in scale between the metrics, but also a distortion of angles, and this can be done, for example, by introducing a preferred time direction or a preferred rest frame. The most natural implementation is to add in a spacetime vector field that has a nonzero value at each point in spacetime; in other words, to point in some direction in spacetime. If that direction is chosen to be (on average) the direction that defines the future (forward in time), as opposed to some direction in space, then the preferred frame will be established (13).

Bekenstein (14) recently proposed a fully relativistic theory that included all of these elements: a disformal relation between the geometric and geodesic metrics, a preferred frame, and modified dynamics for the geometric metric. For an appropriate choice of an arbitrary but universal function, his theory could lead to MONDian dynamics on galactic scales. Bekenstein's theory is known as TeVeS, where the T stands for tensor (representing the metric), V for the time-like vector field, and S

for the scalar field responsible for the scale transformation. TeVeS is actually part of a wider class of models that reproduce MOND on astrophysical scales. An alternative subclass of these models, dubbed generalized Einstein-Aether (GEA) theories, include only the time-like vector field and no scalar field (15).

These modifications of Einstein's GR are sufficiently well defined that it is possible to make firm predictions within each model for what should be observed on various astrophysical and cosmological scales. Such theories have interesting properties, but whereas Einstein's original proposal is highly constrained, these more-complex proposals are less so. Inevitably, they involve extra fields that may come to behave very much like dark matter.

Observational Tests and Limitations

With a relativistic theory of modified gravity in hand, it is possible to make a number of predictions on a range of scales. For a start, one can focus on the effect that TeVeS or GEA will have on the gravitational field of compact objects, such as stars or black holes. It has been shown that the atomic spectral lines from the surface of stars will be affected by TeVeS parameters (16), whereas farther out, there may be directly detectable preferred frame effects that will modify the Newtonian orbits of nearby objects (17). On even larger scales, it has been proposed that the difference in flight time between gravity waves and neutrinos from, for example, a supernova can be used as a signature of modified gravity theories (18). As yet, an analysis of millisecond binary pulsars, one of the GR laboratories par excellence, is still lacking.

Relativistic theories of modified gravity make specific predictions about the dynamics of the universe. The TeVeS theory has a particular property: The energy density in the extra fields is always proportional to the energy density of whatever is the dominant form. Furthermore, the constant of proportionality is independent of the initial conditions and dependent solely on the fundamental constants of the theory (19).

These features lead to a tight constraint on the overall energy density in the extra fields—it cannot be more than one-fifth of the contribution from baryons. Thus, unlike dark matter, this energy density is subdominant to the baryonic mass and does not affect the overall expansion rate. Such behavior can be found in other proposals for modified gravity, but it is not generic. It is challenging to find a parameterization replacing CDM (20), but such theories can predict a wide range of cosmological behavior,

from the highly regular to the unstable, leading to accelerated expansion or to contraction on a finite time scale (21, 22).

Much of the recent advance in cosmology has been accomplished through understanding and measuring the statistical properties of the growth and morphology of large-scale structure, through the cosmic microwave background (CMB) and through surveys of galaxies. With relativistic theories of modified gravity, it is now possible to make predictions on the largest scales, and this has been done for a selection of the currently proposed models, in particular for the original TeVeS model and for GEA theories.

Inhomogeneities evolve in a more complex way in these theories than in the case of GR, with two main new qualitative features. First, the extra degrees of freedom drive the initial growth of perturbations; they seem to be a necessary piece of the theory, and there seems to be no other way to seed structure, given the constraints from observations of the CMB on the amplitude of

A preliminary comparison between the TeVeS theory and both the CMB and large-scale structure data indicates that they are roughly compatible (19). There are a few caveats. First, it may be necessary to include a non-negligible amount of massive neutrinos with a mass of a few electron volts. This is also the mass range required by MOND to agree with clusters. It is still unclear whether this is generic (24), but if indeed it is, it may be testable in the near future. Experiments such as KATRIN (the Karlsruhe tritium neutrino experiment) will bring constraints on the mass of the neutrinos to below 1 eV (25).

Second, there is a subtle effect that can emerge on the largest scales. In GR, when most of the matter is nonrelativistic (in the form of atoms or dark matter), perturbations in the metric can be described in terms of one function, which on small scales is the Newtonian potential that gives rise to the inverse square law of gravity. In modified theories of gravity, perturbations in the metric are generally described in terms of two potentials, one

of which is the Newtonian potential (apart from the deviations required to lead to MOND). The difference between the two potentials, also known as gravitational slip, can lead to changes in the growth of structure, large-scale gravitational lensing, and anisotropies in the CMB. For example, it is still unclear whether it is possible to completely match both the CMB data on large and small scales as well as the amplitude of mass fluctuations in galaxy surveys. If one is to boost the small-angle peaks of the predicted angular power spectrum of the CMB so that it can match the data, one runs the risk of introducing large fluctuations on large scales due to the gravitational slip. Furthermore, this effect can suppress the amount of clustering on galactic, cluster, and supercluster scales. So as yet, the comparison between TeVeS, the CMB, and large-scale structure is not conclusive (26), and all the more so for GEA and other modifications of gravity.

The gravitational slip may be the smoking gun for modified theories of gravity. There are, by now, a few suggestions on how it may be detected. The idea is simple: Different

cosmological data probe different combinations of the two gravitational potentials, and by combining them, it may be possible to tease out evidence for the slip. So, for example, a galaxy catalog will be a measurement of the density contrast of the universe and will be directly related to one of the potentials, whereas a map of large-scale flows should probe the other potential.

Measurements of weak lensing will depend on the sum of the two potentials, as will observations of the CMB. By cross-correlating

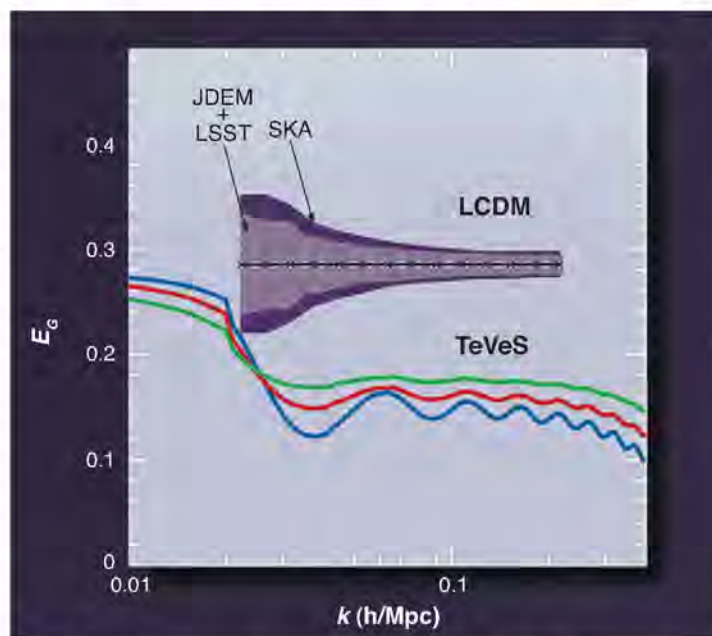


Fig. 3. The normalized cross-correlation spectrum, E_g , between density and weak lensing on large scales, as a function of wave number, k . The points and errors contours are a forecast of what would be expected in a universe with dark matter, a cosmological constant, and Einstein gravity (LCDM) as measured by the Square Kilometre Array (SKA) or by a combination of the Large Synoptic Survey Telescope (LSST) and a possible version of the Joint Dark Energy Mission (JDEM) (27). The colored lines are variants of the TeVeS model of modified gravity. The two different classes of theories are clearly distinguishable.

fluctuations in the baryonic matter density when the universe was 1000 times smaller than it is today. Gravity alone, even (stronger) MONDian gravity, appears to be incapable of growing structure without seeds of such structure that are less coupled to the photons than are baryons (6). TeVeS and GEA avert this conundrum by allowing modes of the new gravitational fields to grow and seed baryonic structure. Effectively, these new degrees of freedom act as dark fields (19, 23).

maps of the CMB with galaxy surveys, or alternatively matching maps of weak lensing with peculiar velocity flows, it should be possible to search for gravitational slip, and, if found, it will give us information about the most-relevant modifications to theories of gravity (6, 27, 28). Relativistic theories of modified gravity can be used to calculate the effects of gravitational lensing and can be tested with the many well-measured gravitational lenses. A notable example is the Bullet cluster, where the baryonic mass, which is usually relatively well traced by hot x-ray-emitting gas, is severely misaligned with the sources of gravitational lensing as inferred from the distorted images of background galaxies (29). A dark matter explanation for the Bullet cluster is that the lensing is centered on two localized accumulations of dark matter—the halos of two colliding clusters that have passed through each other—and the hot cluster gas is interacting as a result of that collision. It would appear difficult to reconstruct such a configuration merely by modifying gravity, but two features of such theories prevent such a simple assessment. The first is the presence of the extra dynamical fields. Because structure is seeded in these models by fluctuations in these extra fields, unsourced by any baryonic fluctuations, these fields clearly are capable of supporting fluctuations that are a source of gravity independent of the baryons. In principle, these gravity-field seeds may evolve into dark field concentrations that interact only gravitationally, become separated from their associated baryons in a collision, and source gravitational lensing as seen in the bullet cluster.

The second feature is that these theories do not satisfy Birkhoff's theorem. As a result, not only is the gravitational field due to a localized concentration of matter that is not unique (and may depend on the history that led to its assembly), but the environment can play a major role in the interactions between two systems. For example, in inferring the dynamics of galaxies within a cluster, it becomes necessary to include the effects from the rest of the cluster, from neighboring clusters, and from any enveloping supercluster (7, 8). Yet, these theories should be predictive, and there have been attempts to study lensing properties of specific galaxies and galaxy clusters in TeVeS and GEA, with mixed results (30, 31). Without including the effect of extra degrees of freedom or the environment, it was found that, in TeVeS, the Bullet cluster would have to be surrounded by massive neutrinos, each with a mass of approximately 2 eV (32). Even with the inclusion of the effects of the extra degrees of freedom, it would be necessary to have some form of dark matter, which could be in the form of neutrinos. This is all hardly surprising, because, as discussed above, MOND requires clusters to have some dark matter. For GEA theories, it is possible, in principle, to reconstruct the geometry and gravitational field of a lens such as the Bullet cluster without any extra matter but with a substantial contribution from the extra degrees of freedom (33). The question of whether the

dynamical evolution of the dark field perturbations leads naturally to such large dark field halos at large time scales remains outstanding.

Although there is nothing intrinsically inconsistent with having the new fields that mediate the modifications of gravity envisioned in MOND act as dark seeds of structure or dark concentrations of gravitational lensing, this necessity detracts from the cleanliness of the original MOND vision: What you see is apparently not what you get, even in MOND. It is therefore much harder to make testable predictions for modified theories of gravity than was already thought, and thus far, these relativistic extensions of MOND remain viable solutions to the problem of missing mass.

Dark Energy and Future Measurements

The problem of missing gravity has been at the forefront of cosmology for many decades. From the moment a proposal was put forward to solve the missing mass problem of galaxies with modified gravity, it was realized that there could be cosmological implications: The acceleration scale, $a_0 \approx 10^{-8} \text{ cm s}^{-2}$, which characterizes the transition between Newtonian and non-Newtonian gravity in MOND, is of the same order of magnitude as cH_0 , where c is the speed of light and H_0 is the expansion rate of the universe today.

Furthermore, the recent discovery that the universe may be accelerating (and not decelerating, as GR predicts if the energy content of the universe is dominated by pressureless, non-relativistic matter) has been taken to imply the existence of an additional dark component of the universe. Dubbed dark energy, it is gravitationally repulsive and can drive the expansion at late times. It may be possible that the accelerated expansion of the universe is instead due to modifications to Einstein's theory of gravity. Some modified gravity theories seek to explain only the accelerated expansion and not the missing mass problem of galaxies and clusters. DGP (Dvali-Gabadadze-Porrati) (34) and (generic) $f(R)$ theories (35) are two that have received widespread attention. However, as for unified models of the dark sector, where an extra component of the energy density of the universe can be both dark matter and dark energy, it is natural to consider models where modifications of gravity can give rise to both the breakdown of Newtonian gravity on galactic scales and to the acceleration of cosmic expansion, as in TeVeS or GEA (15, 21, 36–38).

As argued in the previous section, future surveys may be able to distinguish between theories of modified gravity and GR with dark matter and dark energy (27). A survey of galaxies [such as those that will be done by the Joint Dark Energy Mission (39) or the Square Kilometre Array (40)], combined with a measurement of lensing on large scales [such as those proposed by the Joint Dark Energy Mission or the Large Synoptic Survey (41)], should be able to clearly pick out the signature of gravitational slip of a theory such as TeVeS (Fig. 3). Furthermore, this would be on

scales for which many of the issues that complicate predictions in the case of quasi-isolated systems, such as clusters, would not come into play.

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Population Structure Mediates Sexual Conflict in Water Striders

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In social animals, exploitative strategies often create a paradox in which the most detrimental strategy for the group is also the most successful strategy for individuals within the group. This is often referred to as the tragedy of the commons, an outcome resulting from the unlimited access of individuals to a finite resource, such as herdsman sharing a common pasture. There is always incentive for individuals to add another animal to the herd even as the commons is being overgrazed (1, 2). Although this framework is usually applied to resource consumption, it also applies to the overexploitation of females as a shared mating resource (2, 3).

Just as the overexploitation of resources can lead to local extinction, the overexploitation of females by harmful males can yield similar consequences (4). Laboratory experiments on sexual conflict often confine individuals to isolated groups. This removes the possibility that selection may counter sexual conflict as might be observed in a more natural multigroup population structure.

We measured the fitness consequences of male mating strategies in a single group versus a multi-

group population where variation among groups was caused by the free movement of individuals. We tested the same individuals in both treatments, providing a demonstration of the effect of population structure on the costs and benefits of sexual conflict. The water strider *Aquarius remigis* provides a model species well known to engage in sexual conflict (5) and inhabit multigroup populations in streams in which individuals disperse between pools (6). Building on previous findings (3), we investigated whether the movement of females in response to local aggression creates distribution patterns of females that favor less-aggressive males in *A. remigis*. The experiments took place in a naturalistic laboratory environment consisting of pools that could be either isolated (no-dispersal condition) or interconnected, allowing free movement among pools (dispersal condition) (7).

When dispersal between subpools was removed, the levels of male aggression (score) and male harassment (mating attempts) were positively correlated with mating success (for aggression score, $R^2 = 0.723$, $F_{29} = 73.265$, $P < 0.001$; for harassment, $R^2 = 0.814$, $F_{29} = 122.451$, $P < 0.001$)

(Fig. 1). Furthermore, individual aggression predicted mating success within the isolated pools (Spearman $r_{30} = 0.893$, $P < 0.001$), suggesting that aggression is favored within groups.

In contrast, when dispersal was permitted, aggression only showed a modest quadratic relationship with male mating success (aggression score, $R^2 = 0.314$, $F_{29} = 5.492$, $P = 0.011$; harassment, $R^2 = 0.254$, $F_{29} = 4.078$, $P = 0.030$). Furthermore, females dispersed away from local aggression because mating attempts accounted for 86.4% of the variance in female dispersal between subpools (Pearson $r_{29} = 0.930$, $P < 0.001$). These findings indicate that free movement in a multigroup population may substantially alter costs and benefits associated with male aggression.

Sexual conflict at the scale of local social interactions is well known (8), but few studies have addressed the counterforces mediating the conflict (3, 9), examining only simple population structures. This makes local social interactions the only interactions observed while also reducing female choice. By providing a naturalistic population structure in which the free movement of individuals determines phenotypic variation within and among groups, we have observed another facet affecting mating success.

Because we used the same individuals in both conditions, we demonstrated the importance of population structure. The population structure that we provided in our experiment does not exactly replicate a natural population structure but plausibly may explain why nonaggressive males persist in natural populations despite their relative disadvantage within each and every local group.

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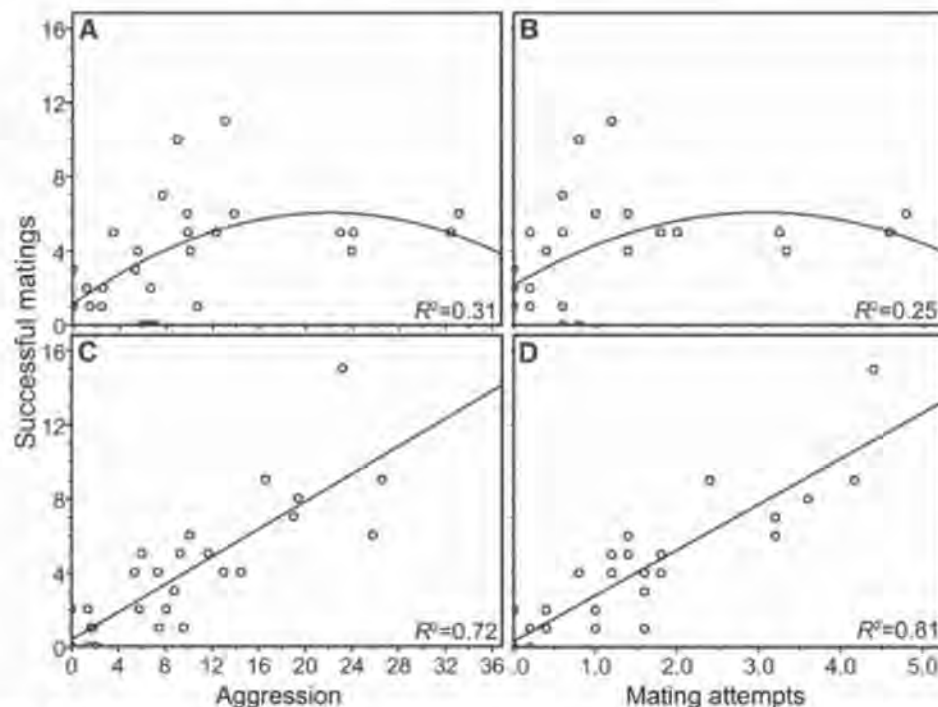


Fig. 1. Differences in mating success of aggressive males between dispersal conditions. In the dispersal condition, male aggression score (A) and harassment (B) had moderate quadratic relationship with the mating success of males. When blocking dispersal, aggression (C) and harassment (D) both strongly predicted the mating success of males.

Genotype Analysis Identifies the Cause of the "Royal Disease"

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DNA variations underlying a specific phenotype or pathology from the past may vanish into history along with carrier individuals or populations. Occasionally, however, these "mutation relics" can be recovered from biological remains and used to reconstruct historical events or solve medical mysteries.

The "royal disease," a blood disorder transmitted from Queen Victoria (1819–1901) to European royal families, contributed to pivotal events in European history and is one of the most striking examples of X-linked recessive inheritance. Although the disease is widely recognized to be a form of hemophilia (a blood clotting disorder), its molecular basis has never been identified (fig. S1). Genealogical analysis suggests that the royal disease mutation was transmitted from Russian Empress Alexandra (granddaughter of Queen Victoria) to her son, Crown Prince Alexei, who suffered from severe bleeding beginning at infancy (1, 2). Our recent DNA analysis revealed that the remains of all members of the Nicholas II Romanov family murdered in 1918 have been found, including the remains of Alexei (3).

We have now analyzed extracts of degraded DNA from the same remains (skeletal bone specimens) by applying multiplex target amplification and massively parallel sequencing (MPS) methods, which allowed us to retrieve and characterize nuclear gene sequences. We used the Alexandra specimens for initial mutation screening of genes for blood coagulation factor VIII, *F8* (26 exons), and

factor IX, *F9* (8 exons), both located on the X chromosome. Mutations in these two genes are the most common cause of hemophilia. Because the DNA samples were limited in quantity and quality, we designed multiple pairs of primers for short amplicons combined into multiplexing polymerase chain reaction (PCR) sets for the *F8* and *F9* genes (4). We analyzed the amplicons from primary or secondary PCR by using MPS in conjunction with conventional sequencing. In parallel, we included MPS of the complete mitochondrial DNA genome as a control for potential contamination and unambiguous identification of the sample (4).

We found no evidence for nonsynonymous missense or small insertion-deletion mutations in either *F8* or *F9* genes in the specimens. However, we detected an A-to-G intronic mutation located three base pairs upstream of exon 4 (intron-exon boundary IVS3-3A>G) in the *F9* gene that could be pathogenic. Typical for a heterozygous carrier, both wild-type and mutant alleles were detected in specimens from Alexandra. The specimens from Alexei contained only the single mutant allele, indicating that he was hemizygous for the mutation, whereas one of his sisters (presumed to be Anastasia) was a heterozygous carrier (Fig. 1 and figs. S2 and S3). Bioinformatics analysis predicts that the IVS3-3A>G mutation at this evolutionarily conserved nucleotide creates a cryptic splice acceptor site (4), which shifts the open reading frame of the *F9* mRNA, leading to a premature stop codon (Fig. 1B).

To evaluate the effect of the mutation on RNA splicing, we used an in vitro exon trap assay in

combination with MPS (fig. S4). We found that 99.98% of transcripts from the mutant *F9* allele were generated by splicing at the mutant site. Hemophilia manifests in a severe form if less than 1% of factor VIII or IX is functional (5).

We conclude that the royal disease is a severe form of hemophilia B, known also as "Christmas disease," caused by a mutation creating an abnormal splicing site in the *F9* gene (4).

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SOM Text

Figs. S1 to S4

Tables S1 to S3

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Fig. 1. The royal disease was likely caused by a point mutation in *F9*, a gene on the X chromosome that encodes blood coagulation factor IX. (A) Partial pedigree of the royal family, showing transmission of the mutation from Queen Victoria to Empress Alexandra and from Alexandra to Prince Alexei, her hemophilic son. Alexei was hemizygous for the

mutation, whereas Alexandra and one of her daughters, putative Grand Duchess Anastasia, were heterozygous carriers. (B) The A-to-G mutation occurs just upstream of exon 4 in the *F9* gene and is predicted to create a new splice acceptor site that could lead to production of a truncated factor IX protein (6). WT indicates wild type.

Hematopoietic Stem Cell Gene Therapy with a Lentiviral Vector in X-Linked Adrenoleukodystrophy

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X-linked adrenoleukodystrophy (ALD) is a severe brain demyelinating disease in boys that is caused by a deficiency in ALD protein, an adenosine triphosphate-binding cassette transporter encoded by the *ABCD1* gene. ALD progression can be halted by allogeneic hematopoietic cell transplantation (HCT). We initiated a gene therapy trial in two ALD patients for whom there were no matched donors. Autologous CD34⁺ cells were removed from the patients, genetically corrected ex vivo with a lentiviral vector encoding wild-type *ABCD1*, and then re-infused into the patients after they had received myeloablative treatment. Over a span of 24 to 30 months of follow-up, we detected polyclonal reconstitution, with 9 to 14% of granulocytes, monocytes, and T and B lymphocytes expressing the ALD protein. These results strongly suggest that hematopoietic stem cells were transduced in the patients. Beginning 14 to 16 months after infusion of the genetically corrected cells, progressive cerebral demyelination in the two patients stopped, a clinical outcome comparable to that achieved by allogeneic HCT. Thus, lentiviral-mediated gene therapy of hematopoietic stem cells can provide clinical benefits in ALD.

X-linked adrenoleukodystrophy [(ALD), Online Mendelian Inheritance in Man database number 300100] is a fatal demyelinating disease of the central nervous system (CNS) caused by mutations of the *ABCD1* gene encoding the ALD protein, an adenosine triphosphate-binding cassette transporter localized in the membrane of peroxisomes. The ALD protein participates in the peroxisomal degrada-

tion of very-long-chain fatty acids (VLCFAs) in oligodendrocytes and microglia, and deficiency of this protein disrupts myelin maintenance by these cells (1). Affected boys enter a phase of active multifocal brain demyelination when they are 6 to 8 years old. Most die before reaching adolescence. Allogeneic hematopoietic cell transplantation (HCT) is the only effective therapy to date, provided that it can be performed at an early stage of brain lesions (2, 3). Beyond a certain stage, demyelination cannot be arrested. The long-term benefits of HCT in ALD are mediated by the replacement of brain microglial cells derived from donor bone marrow myelo-monocytic cells (4, 5). Because the potential of HCT is limited by donor-related constraints and carries a considerable risk of mortality, we reasoned that hematopoietic stem cell (HSC) gene therapy could be an appropriate therapeutic alternative.

Lentiviral vectors, such as those derived from HIV-1, can transduce nondividing cells and allow (both in vitro and in mice) a more efficient gene transfer into HSCs than murine gamma-retrovirus (γ RV) vectors (6, 7). For diseases like ALD, we did not expect that the limitations of γ RV in HSC transduction could be overcome, because the genetically corrected hematopoietic cells have no growth advantage over unmodified cells. However, the greater efficiency of lentiviral vectors in HSC transduction suggested that gene correction might be achieved in a high percentage of HSCs and would therefore result in long-term expression of the

therapeutic gene in all hematopoietic cell lineages of treated patients (8).

ALD gene transfer with lentiviral vectors in vitro has yielded biochemical correction of monocytes/macrophages derived from ALD protein-deficient human CD34⁺ cells (9). In vivo, the transplantation of lentivirally transduced murine ALD Sca-1⁺ cells (a functional equivalent of CD34⁺ cells in humans) into ALD mice resulted in the replacement of 20 to 25% of brain microglial cells expressing the ALD protein 12 months after transplantation (10) (fig. S1). Unfortunately, because the ALD mouse does not develop cerebral demyelination (11), the neuropathological and clinical effects of lentiviral gene transfer could not be assessed in this model. When lentivirally transduced human ALD CD34⁺ cells were transplanted into nonobese diabetic/severe combined immunodeficient (NOD/SCID) mice, the recipient mice showed in vivo expression of ALD protein in human monocytes and macrophages derived from engrafted human stem cells (9). More importantly, human bone marrow-derived cells were shown to migrate into the brain of recipient mice and then differentiate into microglia expressing the human ALD protein (12).

Ex vivo lentiviral-mediated transfer of the *ABCD1* gene into CD34⁺ cells from ALD patients. On the basis of these promising preclinical data and our past experience in treating more than 35 ALD patients by HCT, we initiated a study of lentiviral-mediated HSC gene therapy in two ALD patients who had progressive cerebral demyelination and adrenal insufficiency and who had no human leukocyte antigen (HLA)-matched donor or cord blood for allogeneic HCT. These two patients, aged 7.5 years (P1) and 7 years (P2), had *ABCD1* gene mutations (a large deletion from exon 6 in P1 and E609K mutation in P2), resulting in the absence of ALD protein detectable by immunocytochemistry in fibroblasts and white blood cells. Peripheral blood mononuclear cells (PBMCs) were taken from the patients after stimulation by intravenous injection of granulocyte colony-stimulating factor. After positive selection by an immunomagnetic procedure, CD34⁺ cells were pre-activated ex vivo with a mixture of cytokines (10). The cells were then infected with a replication-defective HIV-1-derived lentiviral vector (CG1711 hALD) expressing wild-type *ABCD1* cDNA under the control of the MND (myeloproliferative sarcoma virus enhancer, negative control region deleted, dl587rev primer binding site substituted) promoter (10). Transduced CD34⁺ cells were frozen so that we could perform replication-competent lentivirus assays (10) before re-infusion. Cryopreserved transduced CD34⁺ cells (4.6×10^6 and 7.2×10^6 cells per kilogram, respectively) were thawed and infused into P1 and P2, after a fully myeloablative conditioning regimen with cyclophosphamide and busulfan. Because lentiviral correction does not provide human or mouse ALD HSCs with a

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selective growth advantage, we used a full myeloablation regimen, as this step would most likely increase the engraftment of transduced HSCs by removal of resident non-transduced HSCs. We observed that 50% (P1) and 33% (P2) of infused CD34⁺ cells expressed the ALD protein, as shown by immunofluorescence (10) 5 days after transduction. The lentivirally encoded ALD protein showed enzymatic activity, resulting in 55% (P1) to 68% (P2) reduction of VLCFA levels in transduced CD34⁺ cells (10). The mean number of integrated provirus copies per cultured CD34⁺ cell was 0.7 and 0.6 for P1 and P2, respectively, 5 days after transduction (10). The procedure was clinically uneventful. Hematopoietic recovery occurred at days 13 to 15 after transplant and was sustained thereafter.

Expression of lentivirally encoded ALD protein in patient's hematopoietic cells after autologous transplantation of gene-corrected CD34⁺ cells. At day +30 after infusion, ALD protein was expressed in 23 and 25% of peripheral blood mononuclear cells from P1 and

P2, respectively (Fig. 1A). In P1, the percentage of PBMCs expressing lentivirally encoded ALD protein decreased to 13% at 9 months after transplant and has stabilized to ~10% at 30 months after transplant (Fig. 1A). In P2, ALD protein expression decreased to 17% at 9 months after transplant and has stabilized to 15% at 24 months after transplant (Fig. 1A). ALD protein expression correlated well with the mean number of integrated vector copies in the same cells (P1, 0.14 copies per cell, 30 months after transplant; P2, 0.20 copies per cell, 24 months after transplant) (10). VLCFA levels were reduced by 20 and 28% in the PBMCs from P1 and P2 at 24 and 20 months posttransplant, respectively.

In both patients, the lentivirally encoded ALD protein was expressed at percentages between 9 and 14% in the different CD14⁺, CD19⁺, CD3⁺, and CD15⁺ peripheral blood lineages, in samples taken 24 to 30 months after transplantation (Fig. 1, B and C). Expression of ALD protein in bone marrow CD34⁺ cells (purity > 99%) went from 20 and 18% (12 months) to 18% (24 months) and

17% (20 months) in P1 and P2, respectively. Similarly, vector-derived sequences were present in 17.3% of colony-forming units–granulocyte-macrophage (CFU-GM) in P1, 24 months after HSC gene therapy, indicating effective gene transfer into common myeloid progenitors with long-term engraftment capacity.

Longitudinal analysis of lentiviral vector insertion and clonal hematopoiesis after autologous transplantation of gene-corrected CD34⁺ cells. The clonal distribution of gene-modified cells in vivo was studied prospectively by a large-scale analysis of lentivirus insertion sites (ISs) with high-throughput 454 pyrosequencing of linear amplification–mediated polymerase chain reaction (LAM-PCR) amplicon (13). Clonal contributions to patient cells were visualized as different size bands representing the multitude of ISs (Fig. 2, A and B). The sensitivity of LAM-PCR combined with next generation sequencing permits the determination of entire insertion repertoires and thereby allows the physiology and kinetics of hematopoietic repopulation to be studied in detail. For LAM-PCR, we applied a combination of restriction enzymes according to an algorithm that allows the amplification of sequences covering all known genomic sequences. LAM-PCR on >98% enriched CD14⁺, CD15⁺, CD3⁺, C19⁺, and bone marrow CD34⁺ cells revealed a high number of distinct ISs, indicating a consistently polyclonal distribution of lentivirally corrected hematopoietic cells over time (Fig. 2, A and B). A total of 2217 (P1) and 1380 (P2) unique LAM-PCR amplicons obtained from patient cell samples before (P1, 501; P2, 484) and after transplantation (P1, 1719; P2, 900) were unequivocally mapped to specific positions in the human genome (14). Collision between samples may arise from the combination of exquisitely sensitive LAM-PCR with deep-sequencing technologies, creating false-positive sequence reads. Collision reads were excluded with the use of a predefined algorithm (10). Typical for lentiviral integrants, insertions were distributed mainly in gene coding regions (P1, 72.71%; P2, 75.80%), without a particular preference for transcriptional start sites, and frequently occurred in chromosomes harboring gene-dense regions (fig. S2) (15).

To determine whether HSCs had been transduced, we compared ISs of lentiviral vector in purified lymphoid CD3⁺ and CD19⁺ cells and myeloid CD14⁺ and CD15⁺ cells from P1 and P2 after transplant, as well as in their CFU-GM colonies derived from posttransplant bone marrow CD34⁺ cells. 86 out of 1846 ISs (myeloid, 940 ISs; lymphoid, 906 ISs) in P1 (4.6%) and 12 ISs out of 835 ISs (myeloid, 578 ISs; lymphoid, 247 ISs) in P2 (1.4%) occurred in both lymphoid and myeloid cells (Fig. 2, C and D). To estimate whether sharing ISs between different lineages could be indicative of initially transduced primitive hematopoietic progenitor cells, we calculated *P* values under the null hypothesis that insertions would follow a Poisson

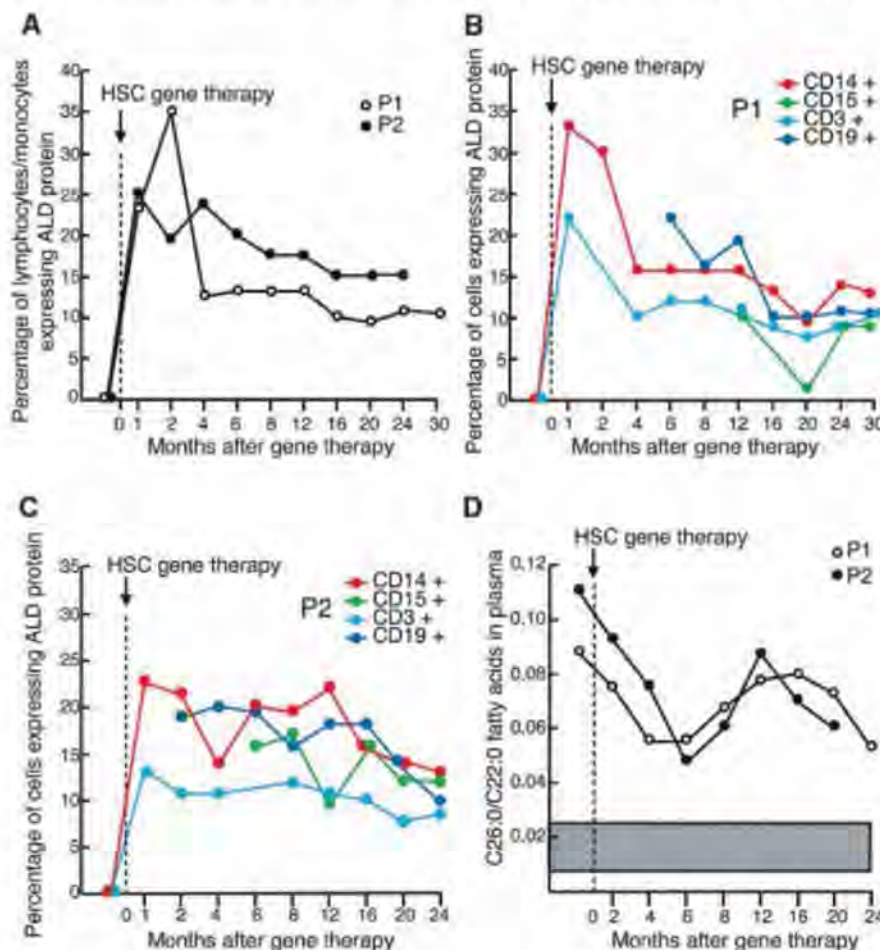


Fig. 1. Gene marking in P1 and P2 after HSC gene therapy. (A) Percentage of peripheral blood lymphocytes and monocytes expressing ALD protein before and after HSC gene therapy. Lymphocytes and monocytes were isolated on a Ficoll gradient and analyzed for the expression of ALD protein after immunostaining with an anti-ALD protein monoclonal antibody (mAb) (12). (B and C) Expression of ALD protein in monocytes (CD14⁺), granulocytes (CD15⁺), T lymphocytes (CD3⁺), and B lymphocytes (CD19⁺) from P1 and P2. Cells were purified on microbeads with appropriate mAbs, and purity (>99%) was checked on a FACS cell sorter. Cells were then analyzed for the expression of ALD protein using an anti-human ALD protein mAb (16). (D) Plasma levels of VLCFAs expressed as C26:0/C22:0 ratio in P1 and P2 before and after HSC gene therapy. The gray band indicates the normal mean $\pm$ SD of C26:0/C22:0 fatty acid ratio.

distribution with expected value $E(10)$. The observed numbers of identities [86 (P1) and 12 (P2), respectively] were at least 22,000 (P1) and 18,000 times (P2) higher than the values expected by chance alone; i.e., they were calculated on the basis of a uniform random IS distribution both over the coding and the non-coding regions ($P = 0$). We used quantitative reverse transcription polymerase chain reaction (RT-PCR) to verify that cross-contamination of CD3⁺ cells and CD14⁺ cells was below 0.2 to 0.4%. Even if one assumes a hypothetical 2% contamination rate by fluorescence-activated cell sorting (FACS), the observed identities in both lineages were still at least 16,000 times higher than the expected values (10). These data indicate that a substantial number of clones with lymphoid and myeloid lineage capability exist and that lentiviral transduction of HSCs had been achieved.

To monitor for early signs of vector-induced dominance of individual clones, we determined the quantitative contribution of individual clones to gene-corrected hematopoiesis by ordering all distinct ISs according to their abundance in every deep-sequencing run on samples containing more than 300 ng of DNA. (Fig. 3, A and B). The retrieval frequency (sequence count) of identical ISs in insertion repertoires obtained by high-throughput sequencing allows a good estimate of clonal contribution, provided that the analyses are performed in samples with comparable clonality, contain a sufficient amount of DNA (13), and an optimized choice of restriction enzymes avoids bias in the amplification of ISs by LAM-PCR. Clonal distribution varied, and no frequent clone re-appeared with an increasing count. No dominance emerged among active hematopoietic clones in the two patients. We also monitored whether the distribution of RefSeq genes with vector insertions changed over time. Insertions affecting the same gene or genomic region in two or more individual cell clones, termed common integration sites (CISs), can indicate that insertion in this particular locus might enhance clonal engraftment, survival or proliferation. No significant difference existed after sample-size adjustment in the proportion of CIS in cells sampled after re-infusion (P1, 30.71%; P2, 15.74%) compared with those sampled before re-infusion [P1, 8.58%; P2, 9.30%] (fig. S3).

We analyzed the gene classes targeted by the lentiviral vector using ingenuity pathway analysis. We found individual gene classes enriched in engrafted cells, but these classes differed between P1 and P2 (fig. S4). Although certain CIS or changes in gene-class enrichment might be related to vector insertion, these findings are currently without known biological or clinical relevance and will be monitored in the molecular follow-up of our patients.

Neuroradiological and neurologic outcomes in ALD patients treated by HSC gene therapy. Before HSC gene therapy, the magnetic resonance imaging (MRI) scan of P1 showed an



Fig. 2. Polyclonal hematopoietic repopulation after autologous transplantation of gene corrected CD34⁺ cells and identical integration sites in myeloid and lymphoid cells in P1 and P2. (A and B) LAM-PCR analysis of ex vivo transduced CD34⁺ cells before transplantation, as well as FACS-sorted cells and colonies at various months (M) after transplantation derived from P1 (A) and P2 (B). CFU, colony forming unit; BM, bone marrow; IC, internal control derived from the annealing of the LAM-PCR primers to the 5'LTR sequences. (C and D) Identical insertion sites found in myeloid (CD14⁺, CD15⁺, CFU-GM) and lymphoid (CD3⁺, CD19⁺) sorted cells in P1 (C) and P2 (D). Light blue, IS identified in myeloid cells at the same timepoint; medium blue, IS identified in myeloid and lymphoid cells at the same timepoint; dark blue, IS identified in lymphoid cells at the same timepoint. See table S1 for a detailed overview of identical IS found in the different sorted cell fractions over time.

abnormal hyperintensity signal that reflected demyelination of pyramidal tracts within the brain stem, pons, internal capsule, and the periventricular frontal white matter, scored at 2.25 (maximum score = 34 points) (16) (Fig. 4A). Enhanced gadolinium contrast indicated that the demyelinating lesions were inflammatory and disrupted the blood-brain barrier (fig. S5). Contrast enhancement of demyelinating lesions disappeared completely 12 months after transplant (fig. S5). Demyelinating lesions continued to extend into the frontal white matter up to month 14 (demyelination score of 6.75), but since then have remained unchanged (Fig. 4A). Before HSC gene therapy, P2 had more extensive demyelinating lesions (Fig. 4B) than P1, with abnormal hyperintensity signal in the splenium of the corpus callosum, the white matter of parieto-occipital lobes, and the auditory pathways scored at 7 (Fig. 4B). Gadolinium contrast enhancement disappeared 9 months after transplant. A minimal contrast enhancement had reappeared in P2 16 months after transplant at the anterior edge of the left parietal white matter lesions, but it has not been detected since then (fig. S5). Lesions extended into the posterior parietal white matter up to 16 months after gene therapy but have remained stable since then (Fig. 4B). The hyperintensity signal involving the auditory pathways disappeared completely, indicating reversal of demyelination, a process that does not occur spontaneously in ALD. Thus, the demyelinating score of P2 remained at 7, as it was before gene

therapy, despite the extension of lesions in the parietal white matter. These results are in sharp contrast with the continuous progression of cerebral demyelination in untreated ALD patients (Fig. 4C), but they are similar to what is typically observed after allogeneic HCT (2, 3). During the first 12 to 24 months after HCT, a brain MRI usually shows a progression of the demyelinating lesions, aggravating the demyelination score by 5 to 6 points out of 34 (3). The demyelinating lesions then stabilize or even reverse, as observed here, with no further changes for decades (2, 3).

The neurologic outcomes of P1 and P2 were also reminiscent of successful allogeneic HCT (3). In untreated ALD patients, the decline of performance and verbal functions is inevitably continuous and devastating during the first 2 years after the onset of inflammatory demyelinating lesions (1). In 40% of ALD patients treated by allogeneic HCT, an initial decline of performance abilities without changes in verbal abilities is followed by stabilization (3). Before HSC gene therapy, P1 had normal neurologic examination and normal verbal intelligence (quotient of 108) but moderate nonverbal performance disability (quotient of 99). After gene therapy, his verbal intelligence has remained unchanged (quotient of 104). An initial decline in nonverbal performance (quotient of 74) has stabilized. He developed muscle weakness on the right side of the body at month 7, which then started to improve until nearly complete regres-

sion by month 14 after transplant. The motor and cognitive functions of P2 were normal before and remained so after HSC gene therapy (verbal and performance of 103 and 111, respectively, 20 months after gene therapy), with the exception of a persistent defect in the lower quadrants of the visual field that appeared 14 months after transplant and has remained stable thereafter. The myeloablative conditioning regimen is unlikely to have contributed to these clinical benefits. Of four patients showing a failure or a marked delay to engraft after this conditioning regimen in our allotransplantation series, all uniformly developed devastating progression of cerebral demyelination and cognitive decline.

Plasma VLCFA levels of ALD patients decrease by ~55% after allogeneic HCT (3), mostly reflecting replacement of liver macrophages by donor-derived cells. Plasma VLCFA levels were reduced by 39% in P1 and 38% in P2, 24 and 20 months after the transplant, respectively (Fig. 1D). Considering that liver macrophages and peripheral blood monocytes originate from the same myeloid precursors, this correction of plasma VLCFA was greater than expected, given that only 13 to 14% of peripheral blood monocytes expressed the ALD transgene in P1 and P2. Similar overcorrection of VLCFA accumulation was observed in PBMCs and transduced CD34⁺ cells from P1 and P2, possibly reflecting ALD protein overexpression or a preferential engraftment of corrected extravascular monocytes/macrophage progenitors early after infusion.

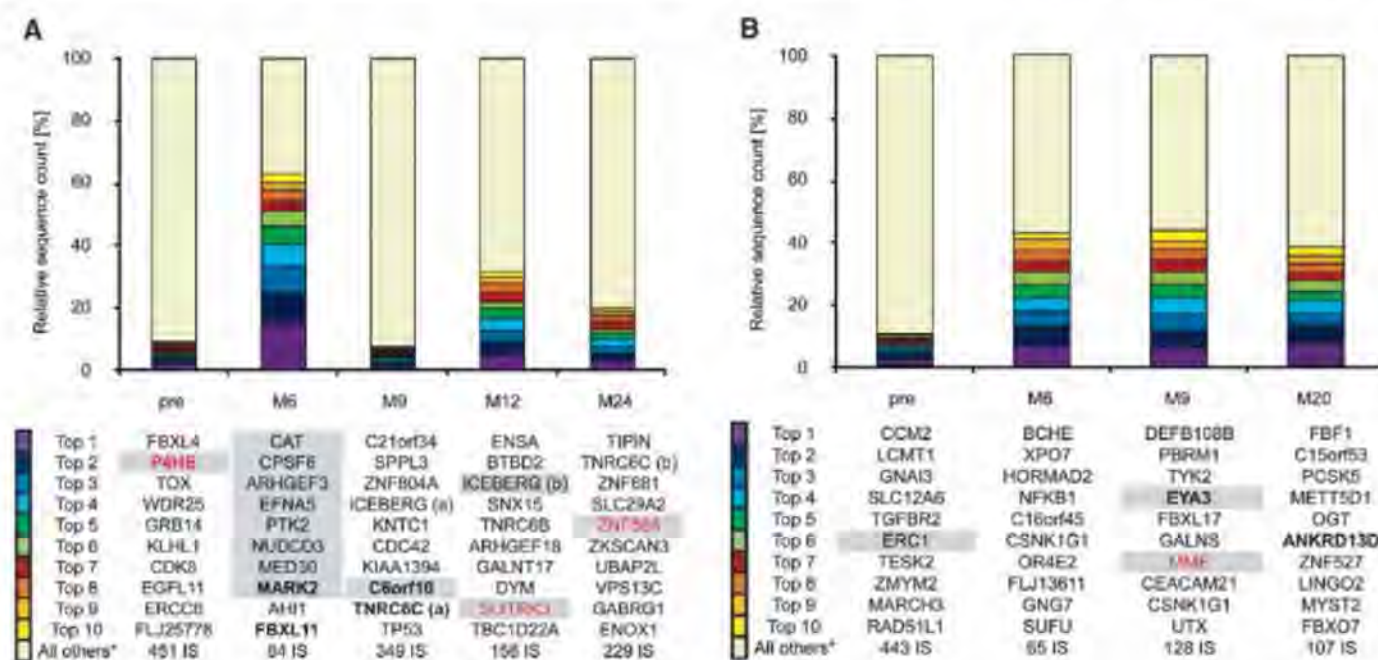


Fig. 3. Retrieval frequency (relative sequence count) of individual integration sites in sequenced polyclonal cell samples from P1 (A) and P2 (B). The quantitative clonal contribution of individually gene corrected cells was assessed by counting identical integration site sequences after 454 pyrosequencing of LAM-PCR amplicons. The relative contribution of individual amplicons is given as the percentage of all insertion flank sequence reads encountered in the particular sample. The 10 most frequent ISs are ranked from 1 to 10, according to frequency. None of the most frequent ISs re-appeared or were found to

contribute a large or growing portion of gene-modified cells to the circulation over extended periods of time. There was no obvious limitation to the clonal repertoire nor was there a clonal dominance. Genes listed in boldface were associated with multiple ISs in different clones (CISs), those listed in red were associated with ISs in both the lymphoid and the myeloid lineages, and those genes highlighted in gray were associated with ISs at more than one timepoint. All others*, all less frequently encountered genetic locations that harbor ISs in the respective sample analyzed.

Quantitative RT-PCR of PBMCs from P1 and P2 showed that the *ABCD1* transgene was expressed at a four- to fivefold higher level than the endogenous mutated *ABCD1* gene (table S2) (10).

Discussion. Previous genetic correction of human HSCs with a murine γ RV vector was successful only in the setting of immunodeficiency disorders such as adenosine deaminase deficiency and severe combined immunodeficiency–X1 (SCID–X1), in which the transgene conferred a selective growth advantage to lymphocytes derived from transduced HSCs (17–20). Lentiviral vectors based on HIV-1 are potentially superior to γ RV vectors, particularly in short-term transduction protocols that minimize ex vivo cell manipulation, because they can be used to transduce candidate HSCs from patients and because they maintain sustained transgene expression even when the genetically corrected HSCs do not acquire a growth advantage (21, 22). In the present trial, the long-term stability of ALD pro-

tein expression in myeloid cells, as well as the presence over time of identical ISs in both the myeloid and lymphoid lineages, strongly suggest that HSCs have been transduced with the capacity to self-renew and repopulate multiple hematopoietic lineages.

The adverse events that occurred in SCID–X1 patients treated with γ RV vector gene therapy have raised serious concerns about retroviral integration-related mutagenesis and leukemogenesis (23). Transcriptionally active long terminal repeats (LTRs) of retroviral vectors are major determinants of genotoxicity (24), whereas the LTR promoter/enhancer of lentiviral vector used in this study is self-inactivating (SIN) upon transduction. This and other differences in lentiviral and oncoretroviral biology suggest that the risk of insertional mutagenesis by a SIN lentiviral vector may be lower than that with γ RVs and even SIN γ RVs (24–26). In our study, we took advantage of new deep-sequencing technol-

ogies for genome-wide monitoring of lentivirus-marked HSC clonality in the patients. Although we did not detect obvious clonal skewing or dominance in hematopoiesis, a longer follow-up and a larger sample size will be required to verify that the potential for genotoxicity of lentiviral vectors in this application is low.

Before this gene therapy trial, we did not know how many corrected HSCs would need to be infused to achieve clinically relevant neurological benefit, because this crucial issue could not be addressed in the phenotypically normal ALD mouse (11). We only knew that all affected ALD boys who were successfully treated by allogeneic HCT and showed stabilization and/or regression of cerebral demyelination had more than 80% donor-derived engraftment (2, 3). In the current study, long-lasting expression of ALD protein in ~15% of monocytes (CD14⁺), a population of cells that have the same myelomonocytic origin as bone marrow–derived brain microglia (27), was sufficient to obtain comparable neurological benefits after gene transfer into autologous HSCs, probably because ALD protein is overexpressed in microglial cells that derive from transduced CD34⁺ cells (28). Further studies will be needed to test the balance between less toxic partial myeloablation and the need for effective engraftment of transduced HSCs.

The fact that the neurologic benefits of HSC gene therapy in ALD were comparable to those seen with allogeneic HCT supports further testing of this treatment in an extended series of ALD patients with cerebral demyelination who do not have an available HLA-matched donor or cord blood. HSC gene therapy might also be considered as a therapeutic option for adult ALD patients who develop cerebral demyelination, for whom the mortality risk of allogeneic HCT is ~40%. Finally, the recruitment of bone marrow–derived cells to CNS macrophages/microglia may provide a new avenue for cell-based gene therapy in other genetic and multifactorial CNS diseases.

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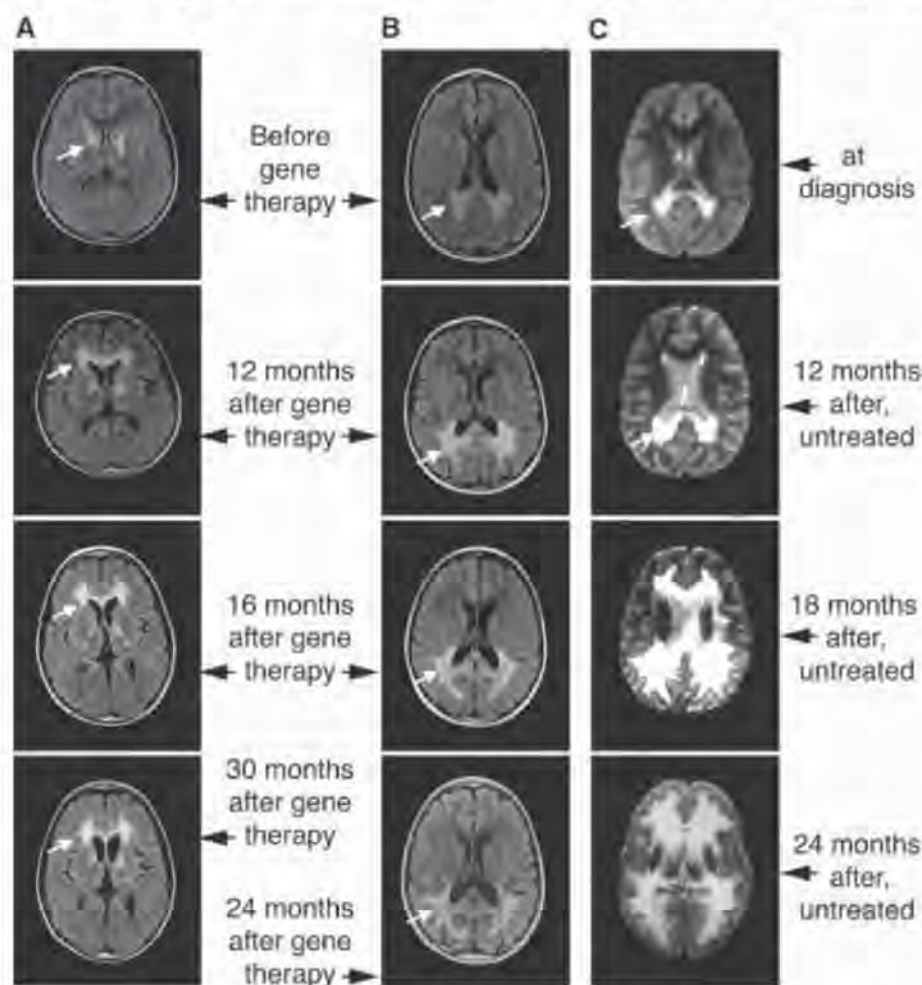


Fig. 4. Brain MRIs from P1 (A) and P2 (B) before and after gene therapy. (A) Fluid attenuated inversion recovery (FLAIR) sequences show hypersignal involving the pyramidal tracts within the internal capsules (white arrows) and periventricular frontal white matter of P1 before gene therapy. These lesions extended into the frontal white matter up to 14 months after HSC gene therapy and then did not show progression. (B) In P2, FLAIR sequences show hypersignal involving the corpus callosum, the parieto-occipital white matter (white arrows), and the auditory pathways before gene therapy. These lesions extended into the white matter of posterior parietal lobes up to 16 months after gene therapy and then stabilized with no further progression. (A and B) A mild dilation of frontal (P1) or occipital (P2) horns of ventricle is present 20 to 24 months after gene therapy. (C) Progression of cerebral demyelinating lesions in an untreated 8-year-old ALD patient (demyelination score at 26, 24 months after diagnosis).

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Supporting Online Material

www.sciencemag.org/cgi/content/full/326/5954/818/DC1

Materials and Methods

SOM Text

Figs. S1 to S5

Tables S1 and S2

References

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Three-Color Entanglement

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Entanglement is an essential quantum resource for the acceleration of information processing as well as for sophisticated quantum communication protocols. Quantum information networks are expected to convey information from one place to another by using entangled light beams. We demonstrated the generation of entanglement among three bright beams of light, all of different wavelengths (532.251, 1062.102, and 1066.915 nanometers). We also observed disentanglement for finite channel losses, the continuous variable counterpart to entanglement sudden death.

Since Schrödinger defined entanglement as “the characteristic trait of quantum mechanics” (1), much study has been devoted to its understanding. Possible applications to information tasks such as storage, computation, and communications have generated a large amount of research (2). Quantum computing is expected to be more efficient than its classical counterpart, and there are quantum algorithms (3) that greatly surpass the best classical ones. Quantum communications can, in principle, provide absolute security (4). Nevertheless, many technological challenges remain, because the quantum resources are fragile and undergo decoherence from their inevitable interactions with the surrounding environment (5). Furthermore, many fundamental issues regarding the nature and dynamics of entanglement remain to be understood (6).

A variety of physical systems are currently under investigation to perform the envisioned information tasks (7–12). Because each one has

its advantages and disadvantages, it is likely that several of them will be combined to perform reliable quantum information tasks. Light, in view of its high speed and weak interaction with the environment, is a strong candidate to convey quantum information by using quantum teleportation (13, 14). To connect such different physical systems (typically lacking a joint resonance) at the nodes of a quantum network (15), however, different frequencies of light will be necessary. For two such beams, entanglement has already been demonstrated, ranging from small frequency differences (16, 17) up to one frequency being twice the other (18). It is important to go beyond just two beams for multi-mode networks. Our demonstration used three field modes with different wavelengths.

Our system is an optical parametric oscillator (OPO), which consists of a nonlinear optical crystal inside a cavity, so that the fields are fed back into the system. Light that is incident on the crystal undergoes parametric down-conversion, a process whereby an incident photon is converted into a pair of longer-wavelength photons, fulfilling energy conservation $\omega_0 = \omega_1 + \omega_2$, where the ω_i ($i = 0, 1, 2$) values are the angular frequencies of the incident light and of the two generated photons, respectively. Momentum is also conserved, corresponding to the phase-

matching condition. Owing to the cavity feedback, down-converted photons can be emitted in occupied field modes, a process known as stimulated emission, with increasing probability as the number of photons in the mode grows, thus providing gain. When the pump laser power is increased, the gain overcomes the losses and the system oscillates. Above this oscillation threshold, tripartite entanglement has been predicted (19). Down-converted photons are produced in pairs, yielding strong intensity correlations among the twin beams. To produce twin photons, a pump photon must be annihilated; thus, anti-correlations are expected between the reflected pump intensity and the sum of twin beams' intensities. The frequency constraint translates into a constraint for the phase variations (or fluctuations) of the three fields. The twins' phase fluctuations should be anticorrelated, and their sum should be correlated to the pump's phase fluctuations. One of the criteria for analyzing tripartite entanglement (20) is written directly in terms of these correlations, as sums of variances V involving the three fields.

$$\begin{aligned} V_0 &= \Delta^2 \left(\frac{p_1 - p_2}{\sqrt{2}} \right) + \Delta^2 \left(\frac{q_1 + q_2}{\sqrt{2}} - \alpha_0 q_0 \right) \geq 2 \\ V_1 &= \Delta^2 \left(\frac{p_0 + p_1}{\sqrt{2}} \right) + \Delta^2 \left(\frac{q_0 - q_1}{\sqrt{2}} - \alpha_2 q_2 \right) \geq 2 \\ V_2 &= \Delta^2 \left(\frac{p_0 + p_2}{\sqrt{2}} \right) + \Delta^2 \left(\frac{q_0 - q_2}{\sqrt{2}} - \alpha_1 q_1 \right) \geq 2 \end{aligned} \quad (1)$$

It suffices to violate two of these inequalities to demonstrate tripartite entanglement. The p_i ($i = 0, 1, 2$) represent the amplitude quadratures of the fields, the q_i ($i = 0, 1, 2$) stand for their phase quadratures, and the α_i ($i = 0, 1, 2$) values are free parameters, chosen to maximally violate the inequalities. Phase and amplitude

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quadratures of each field do not commute, leading to a minimum uncertainty product, which characterizes the standard quantum limit (SQL). Variances in Eq. 1 are normalized to the SQL.

Above the oscillation threshold, bright, narrow-band, and frequency-tunable twin beams are emitted by the OPO. Their quadrature fluctuations are measured by reflecting them off nearly resonant empty analysis cavities (21) before detection on high-quantum efficiency photodiodes (Fig. 1). Any quadrature of any given field can be measured by scanning the resonance frequency of the respective analysis cavity. In this way, the full three-field covariance matrix of the system, with terms of the form $\langle \delta X_i(\Omega) \delta X_j(-\Omega) \rangle$ and $\langle \delta X_i(\Omega) \delta Y_j(-\Omega) \rangle$ (where X and Y represent the field amplitude and phase quadratures; $i, j = 0, 1, 2$; and Ω is the analysis frequency), is directly measured (22). Thermal phonons that are present in the nonlinear crystal generate excess phase noise and hinder the quantum correlations, as recently described (23). Thus, we cooled the crystal to -23°C .

For Gaussian states, the complete information is available from the mean values (first-order moments) and the covariance matrix (second-order moments), of which only the latter is relevant for entanglement properties. By inspecting higher-order moments (up to 10) from the measured photocurrents, we verified that within the experimental precision, the three-field state from the above-threshold OPO is indeed Gaussian. We can thus test the entanglement using a necessary and sufficient criterion for Gaussian states: the positivity under partial transposition (24, 25). If one party is separable from the rest, the full density matrix remains positive under partial transposition with respect to that party. Partial transposition for Gaussian states is equivalent to inverting the sign of the quadrature of one of the fields (24). The positivity is checked by evaluating the symplectic eigenvalues of the partially transposed matrix. The state is separable if and only if all the symplectic eigenvalues are greater than or equal to 1. This criterion is necessary and sufficient for all $1 \times N$ decompositions of Gaussian states (25), where $N + 1$ is the total number of entangled modes. In the tripartite scenario, the three possible 1×2 partitions have to be tested.

To demonstrate the full three-field inseparability (Fig. 2), we characterized the OPO for several values of the pump power relative to the threshold power ($\sigma = P/P_{\text{th}}$). For each value of σ , the covariance matrix was directly measured, and the smallest symplectic eigenvalue under partial transposition for each field was evaluated, as well as the standard deviations of the eigenvalues (21). Full inseparability was demonstrated for σ ranging from 1.1 to 1.6 and for crystal temperatures below -10°C . In terms of the inequalities of Eq. 1, which provide only a sufficient criterion, entanglement was also verified: $V_0 = 1.35 \pm 0.02$, $V_1 = 1.93 \pm 0.02$, and $V_2 = 1.93 \pm 0.02$ for $\sigma = 1.24$. This represents the direct generation of continuous-variable entan-

glement between more than two subsystems, and we took advantage of this approach by entangling fields of different wavelengths. Continuous-variable multipartite entanglement is classified in terms of full or partial inseparability (26). The phonon-induced phase noise in the OPO enables transitions from full inseparability to partial inseparability (the pump field becomes separable) just by tuning a single parameter, the crystal temperature.

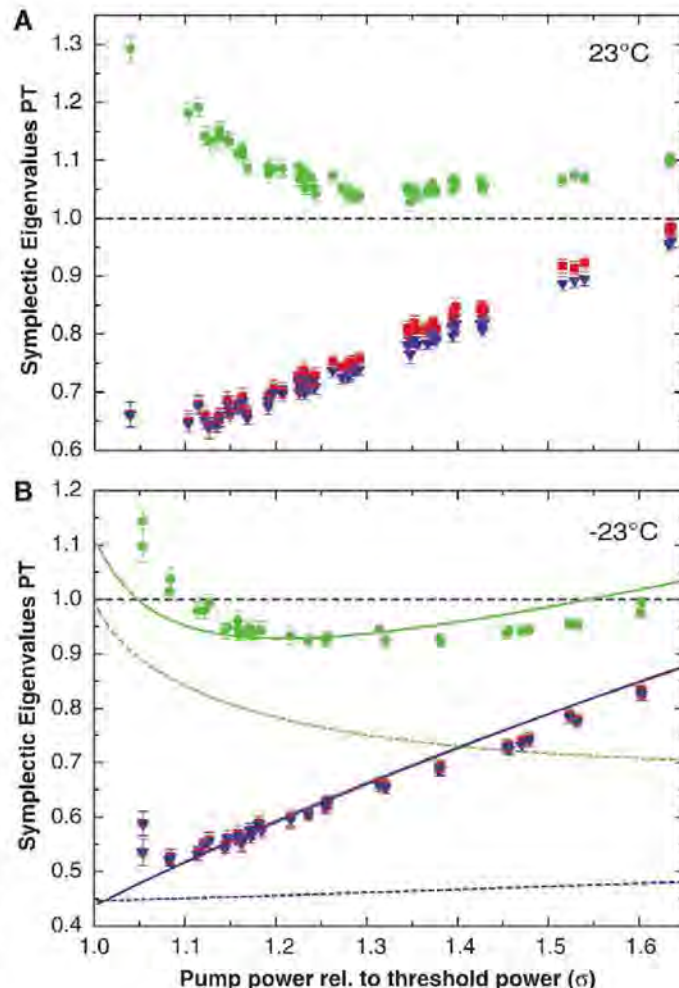
Because losses are a concern in communications systems, it is important to assess the robustness of the generated tripartite entangle-

ment. We investigated the dependence of the symplectic eigenvalues on linear losses imposed on the twin beams by placing variable attenuators immediately in front of the photodetectors (we verified that pump beam losses had little effect). As expected, noise terms in the covariance matrix linearly approach the SQL and correlations tend to zero, as losses increase (21). This is also an important verification that the detection system is working properly. Although a squeezed state remains squeezed under finite linear losses, we found that tripartite entanglement behaves differently: We observed disentanglement of the

Fig. 1. The OPO (blue, center) is pumped by a coherent light source, producing three light fields of different wavelengths (green, red, and orange arrows). Entanglement verification is realized by using one analysis cavity for each beam to access all entries of the full covariance matrix. The golden rings illustrate the tripartite entanglement among the optical fields.



Fig. 2. Symplectic eigenvalues, extracted from the measured covariance matrices, are plotted as functions of $\sigma = P/P_{\text{th}}$. Three eigenvalues are plotted, each corresponding to partial transposition with respect to one of the beams. (A) Measurements made at $+23^\circ\text{C}$. We observe that at room temperature, v_0 (transposition of the pump, green circles) is greater than 1, whereas v_1 (transposition of signal, red squares) and v_2 (transposition of idler, blue triangles) are smaller than 1 for a large range of σ values. Thus, at room temperature only bipartite entanglement between the twin beams exists. (B) At -23°C , we also observed v_0 drop below 1, demonstrating the full inseparability of the three fields. The dashed lines indicate the usual theoretical prediction without taking into account the phonon noise. By taking into account the phonon noise (23), we obtain the solid lines, which agree well with the data. Error bars are standard deviations (21).



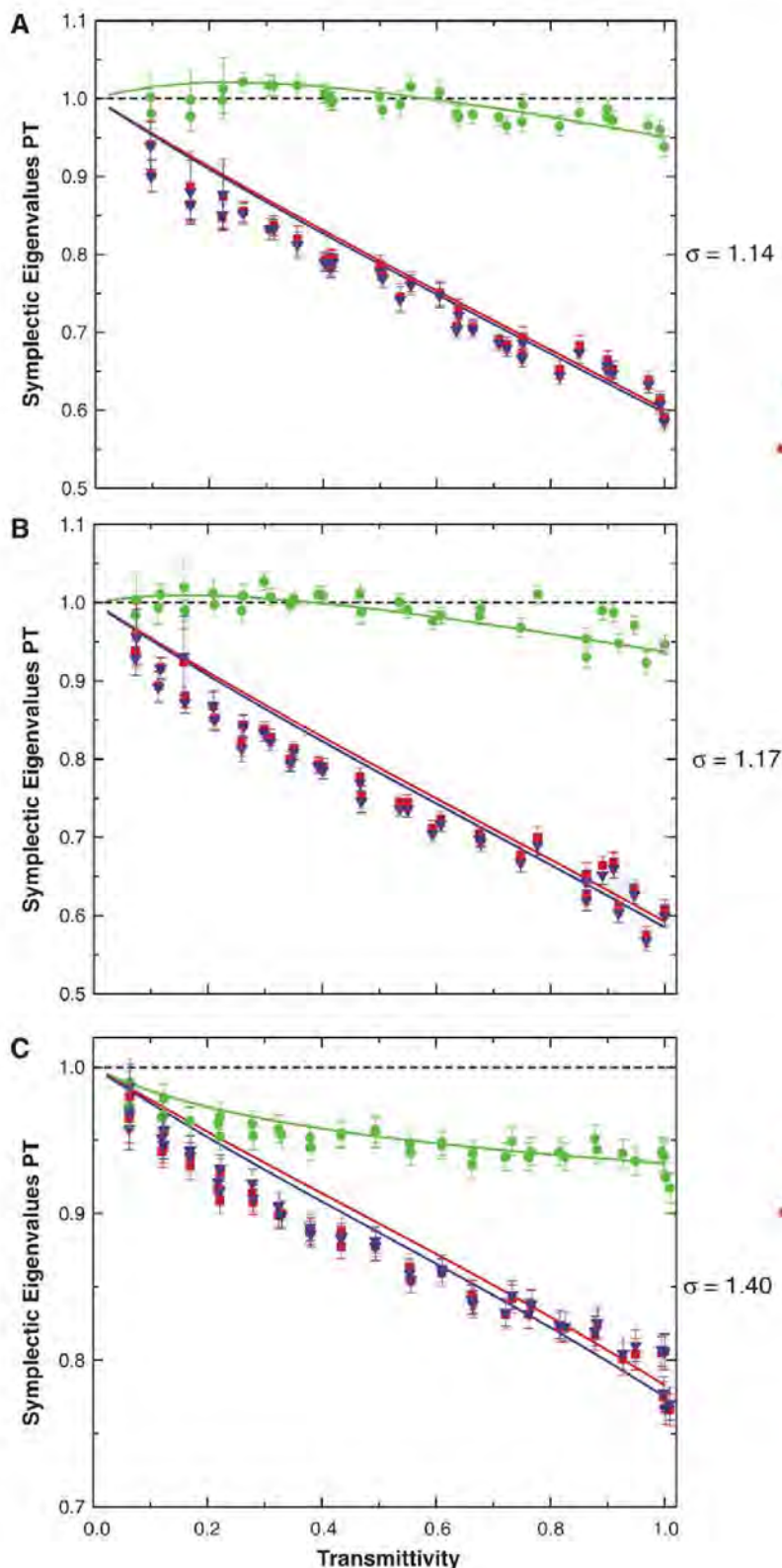
pump field from the twins for finite losses (Fig. 3). This effect is reminiscent of so-called entanglement sudden death (6, 27), which is observed in discrete-variable systems and predicted to occur in continuous-variable systems as well (28, 29). For a pair of entangled qubits, each interacting with an independent environment, even though single-qubit coherence decays exponentially, entanglement can be lost at a finite time. Like-

wise, even though squeezing of individual beams tends only asymptotically to the SQL as a function of losses, we already observed disentanglement for finite losses. This could be harmful for applications to quantum communications, such as in information networks. On the other hand, it is not present for all values of σ , as can be seen in Fig. 3; this is an important result for our system. The robustness of entanglement with

respect to channel losses depends on σ , indicating that the entangled states are not of the same nature, even though they are always Gaussian. The phonon noise present in the entangled state that exits the OPO cavity certainly plays a role in this process. The theory neglecting this noise does not predict disentanglement.

The tripartite entangled state demonstrated here presents a fundamental feature—disentanglement

Fig. 3. Investigation of disentanglement for finite losses. Color conventions are the same as in Fig. 2. In (A) ($\sigma = 1.14$) and (B) ($\sigma = 1.17$), we observe that the pump beam becomes separable from the twins for finite losses (transmittance near 0.6 and 0.4, respectively); on the right-hand side, the situation is sketched. Because of the variable linear losses imposed on the twin beams (beam-splitter losses), entanglement of the pump beam is lost, as indicated by the golden rings. In (C) ($\sigma = 1.40$), there is no disentanglement for finite losses and the entanglement signature is monotonically reduced, yet all three fields remain inseparable until total loss, as sketched on the right-hand side. Data were obtained at -10°C . The solid curves were extracted from the covariance matrices that were obtained with no attenuation by calculating the effect of losses on each of their elements (21).



for finite linear losses—that should be observable in other continuous-variable systems as well. This serves as a reminder that much is still unknown about the nature and dynamics of quantum entanglement. The relatively small violations of the entanglement criteria found here can be improved by better OPO cavity optics and by further cooling of the nonlinear crystal. Another possibility that is currently under consideration is to change the wavelengths. The OPO pump field can be at 780 nm, so as to interact resonantly with Rb atoms, generating output fields at wavelengths close to 1560 nm. Quantum information stored in an atomic sample can thus be transferred to a wavelength that is suitable for propagation in low-loss optical fibers. Future quantum information networks may connect systems of different natures by multicolored entangled light.

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Materials and Methods

Fig. S1

References

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Electronic Structure Controls Reactivity of Size-Selected Pd Clusters Adsorbed on TiO₂ Surfaces

William E. Kaden, Tianpin Wu, William A. Kunkel, Scott L. Anderson*

The catalytic activity of metal clusters of different sizes adsorbed on oxide surfaces can be explored systematically by using model catalysts. We studied the temperature-programmed reaction of CO with O₂ catalyzed by Pd clusters (Pd_n, for $n = 1, 2, 4, 7, 10, 16, 20$, and 25) that were size-selected in the gas phase and deposited on rutile TiO₂(110). X-ray photoemission spectroscopy revealed that the Pd 3d binding energy varied nonmonotonically with cluster size and that the changes correlated with strong size variations in CO oxidation activity. Taking final-state effects into account, low activity was correlated with higher-than-expected Pd 3d binding energy, which is attributed to a particularly stable valence electronic structure; electron transfer from the TiO₂ support to the Pd clusters also occurs. Ion scattering shows that small clusters form single-layer islands on the surface and that formation of a second layer begins to occur for clusters larger than Pd₁₀.

Heterogeneous catalysts often consist of small metal clusters, adsorbed on metal oxides. Because these clusters can vary widely in size, it can be difficult to sort out the extent to which changes in reactivity with cluster size predicted by theory (1–3) are caused by variations in electronic structure or in geometric effects (the number and type of reactive sites). During the past decade, model catalysts consisting of small mass-selected clusters (one to 30 atoms) deposited on oxide surfaces have been used to demonstrate that catalytic activity can

depend strongly on cluster size (4–9). However, the systematic interpretation of size trends has been limited by the lack of experimental probes of sample structure. Here, we present an example in which reactivity is clearly correlated with the electronic structure of the supported metal: in this case, CO oxidation over planar Pd_n/TiO₂ (110) model catalysts, with $n = 1, 2, 4, 7, 10, 16, 20$, and 25. The changes in electronic structure are determined by both inherent size effects and by cluster-bonding to the oxide support.

The small cluster size range is where the largest effects of cluster size are expected, but recent work has demonstrated that such small clusters can dominate catalytic activity, even when most of the catalytic metal is present in the form of much larger particles (10, 11). CO oxidation has been studied extensively over Pd/metal-oxide

(12, 13) and single-crystal surfaces (14), both because it is relatively simple and because it is important in pollution remediation.

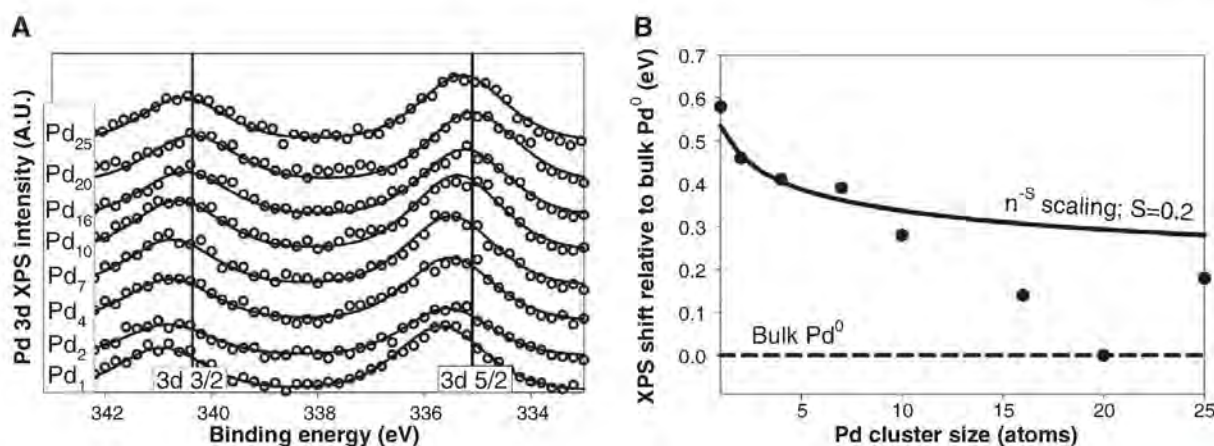
The experimental protocol is detailed in (15). Briefly, Pd/TiO₂(110) samples were prepared and studied by the deposition of mass-selected Pd_n⁺ on clean, vacuum-annealed rutile TiO₂(110) at an energy of 1 eV per atom. The TiO₂(110) support is commonly used in model catalyst studies and has well-characterized properties (16–21). The support is conductive enough to neutralize the initial ion charge, and the 1 eV-per-atom energy previously has been shown to result in the landing of intact clusters (that is, the sample structure is determined by the chemical interaction of the clusters with the support, not impact energy) (22, 23). All samples contained the same total Pd concentration (1.53×10^{14} atoms per cm² = 0.1 monolayer equivalent), with the only difference being cluster size. Deposition times were 10 to 30 min with a background pressure of 1.5×10^{-10} torr. X-ray photoemission spectroscopy (XPS) was used both to verify the Pd coverage on the samples and to probe the Pd electronic structure. The as-deposited Pd 3d XPS as a function of cluster size is shown in Fig. 1A. The vertical lines indicate the binding energies (BEs) (the energy difference between the initial and final states of the photoemission process) observed for bulk Pd, which are consistent with reported values (24). The shift in 3d_{5/2} BE, relative to bulk Pd, as a function of cluster size is shown in Fig. 1B.

Initial state effects, also known as chemical shifts, reflect changes in the electronic structure of the sample. For example, if bonding to the support transferred an electron to the Pd clusters, the Pd BE would shift to lower energy, and if bonding to the support transferred an electron from the Pd clusters, the Pd BE would shift to higher energy. Quantum confinement effects and

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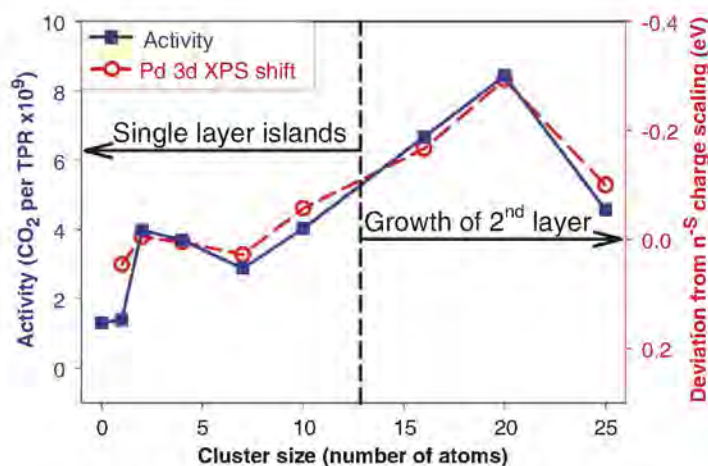
Fig. 1. (A) Pd 3d XPS for as-deposited Pd_n for $n = 1, 2, 4, 7, 10, 16, 20$, and 25 on TiO₂(110). (B) Shifts in the observed Pd 3d peak position relative to the bulk Pd⁰ limit as a function of cluster size. Analysis of binding-energy uncertainty (± 0.02 eV) is described in (15).



changes in metal-metal bonding in small clusters have also been proposed as sources of initial state shifts (25, 26). The final state of the photoemission process is an atom with a hole in a core level, and there are several effects that can shift the binding energy, relative to the bulk value. These effects include the decrease, relative to bulk metal, of core-hole screening in small particles and the size-dependent charging energy for clusters on an insulating support. For isolated spherical clusters, this charging energy would scale inversely with radius, or scale as $n^{-1/3}$. Such scaling has been observed for supported noble metals by several groups (27–29). The smooth curve plotted in Fig. 1B shows n^{-S} scaling. It was necessary to reduce the exponent S from 0.33 to 0.2 in order to get the curve to pass through the data for the small clusters ($n^{-1/3}$ scaling from the bulk BE would predict much greater shifts than are observed). This change in scaling is not unexpected because it could result from charge delocalization in the final state to the TiO₂ surface or from deviation of the clusters from spherical shape. The exact form of the scaling is unimportant here; the key point is that in the small size regime, the binding energies fluctuate, and as we show below these fluctuations are strongly correlated with reactivity.

The correlation is shown in Fig. 2. The right-hand axis (inverted scale) shows the deviation of the experimental Pd 3d BEs from $n^{-0.2}$ scaling. The left-hand axis shows the CO oxidation activity, given as the number of CO₂ product molecules desorbing during temperature-programmed reaction (TPR) on each sample. In both cases, the uncertainty in the data was estimated as discussed in (15). The uncertainty in the TPR results was estimated to be $\sim \pm 5 \times 10^8$ CO₂ per TPR, whereas that for the XPS was estimated to be $\sim \pm 0.02$ eV. For TPR, the as-deposited Pd_n/TiO₂ samples were first exposed to 10 Langmuir (L) of ¹⁸O₂ at 400 K (1 L = 10^{-6} torr · s of exposure) and then to 5 L of ¹³CO at 180 K. The sample temperature was then ramped at 3 K/s from 130 to 530 K while we monitored masses of interest (¹³C¹⁶O¹⁸O and ¹³CO) with a differentially pumped mass spectrometer. Activity was essentially identical for TiO₂ (cluster size “0”) and Pd₁/TiO₂, indicating that single Pd atoms are unreactive on this surface. Activity in-

Fig. 2. CO oxidation activity observed during TPR (left axis, solid squares) compared with shifts in the Pd 3d binding energy, relative to expectations from smooth bulk scaling (right axis, open circles), as a function of cluster size. Analysis of uncertainty ($\pm 5 \times 10^8$ CO₂ per TPR) is described in (15). The XPS and TPR data for each cluster size were taken for the same sample.



creased substantially for Pd₂ then declined slowly as the cluster size increased to Pd₇. For larger clusters, activity increased monotonically before dropping again for Pd₂₅. The XPS BE shifts are inversely correlated. Clusters with BEs higher than expected from scaling showed suppressed reactivity and vice versa. This correlation is not simply an artifact of the $n^{-0.2}$ scaling that we used. An equally good correlation is obtained using a straight trend line through the data, as shown in fig. S1 (15). Uncorrected TPR results for TiO₂ and Pd_n/TiO₂ ($n = 1, 2, 7$, and 20) are shown in Fig. 3. In plots of desorption of the unreacted ¹³CO (Fig. 3A), the peak at ~150 K remains approximately constant in all cases and is attributed to CO bound at oxygen vacancy defects present in the TiO₂ support (30). The other two peaks, seen only when Pd is present, show variations with cluster size. The low- (~200 K) and high-temperature (~430 K) peaks have roughly equal integrated intensities for reactive clusters such as Pd₂ and Pd₂₀. In contrast, the high-temperature CO desorption feature is smaller for relatively unreactive clusters such as Pd₁ and Pd₇.

The analogous CO₂ desorption profiles (Fig. 3B, collected simultaneously with the CO data in Fig. 3A) illustrate the correlation between CO₂ production (Fig. 3B) and the intensity of the high-temperature CO desorption feature (Fig. 3A) and suggest that it is only this more stably bound CO that is reactive. Although CO oxidation could de-

plete this “reactive” CO component and eliminate the correlation, the reaction had only about 5% yield, even for the reactive CO component (compare the vertical scales in Fig. 3, A and B).

For comparison, Fig. 3A also plots CO desorption from a Pd₂₀/TiO₂ sample that was not exposed to O₂ before CO exposure (Fig. 3A, thin curve). Similar data were obtained for other cluster sizes for CO desorption from unoxidized Pd_n/TiO₂ samples. The 150 K desorption feature was considerably enhanced, which suggests that the 400 K O₂ exposure led to partial healing/blocking of O-vacancy sites on the TiO₂. More importantly, the 200 K feature was essentially unchanged by O₂ pre-exposure, whereas the high-temperature CO desorption feature was substantially reduced by O₂ pre-exposure. This behavior is further evidence that only CO bound to the more stable high-temperature site reacts to form CO₂ product.

The correlation shown in Fig. 2 shows that the Pd electronic structure, as manifest in core-level binding-energy shifts, is strongly correlated with CO oxidation activity. We also used He⁺ ion scattering (ISS) to examine the dependence of sample morphology on cluster size and to look for correlations between activity and geometrical structure. Under our conditions, the ISS spectra are dominated by He⁺ scattering from a single atom in the surface layer (22, 31), and the mass of the surface atom is given by the energy of the scattered He⁺. A spectrum for as-deposited Pd₁₀/TiO₂

Fig. 3. Desorption of unreacted CO (A) and CO₂ products (B) during TPR after 400 K oxidation and 180 K CO exposure to Pd_n/TiO₂ (*n* = 1, 2, 7, and 20) and uncovered TiO₂. The thin curve shown for Pd₂₀ in (A) shows CO desorption from a sample not exposed to O₂. All spectra result from individual experiments.

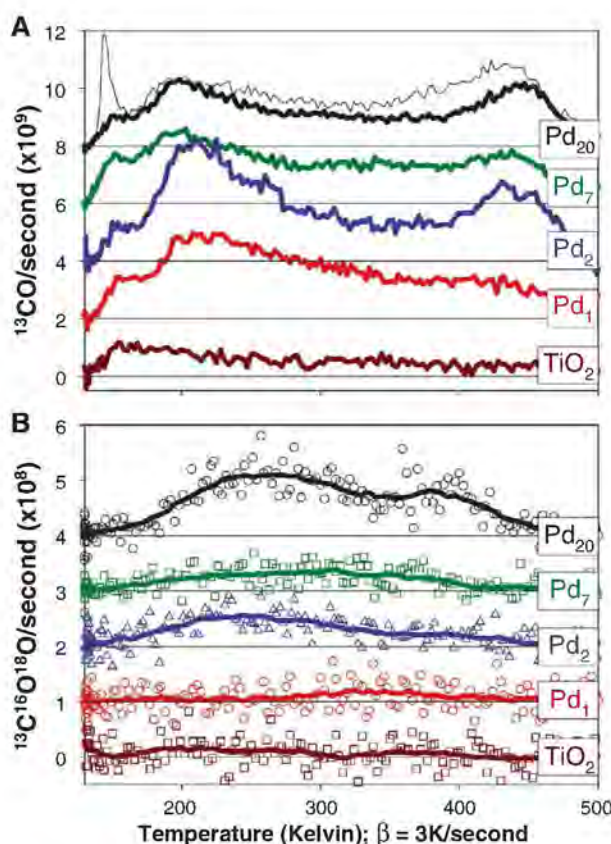
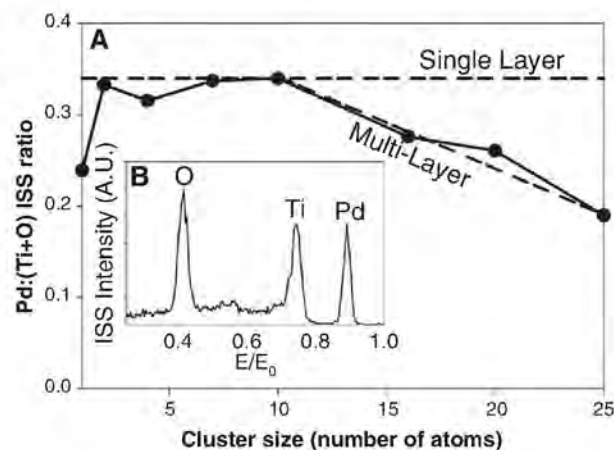


Fig. 4. (A) Ratio of Pd ISS signal to the sum of Ti and O signals as a function of cluster size. Data points represent results for single-sample preparations. (B) A typical ISS spectrum for Pd₁₀/TiO₂(110).



(Fig. 4B) is provided along with the ratio of Pd to Ti + O peak intensities as a function of cluster size in Fig. 4. This ratio is related to the fraction of the surface layer composed of Pd (the dispersion). As long as the Pd is all in the surface layer [two-dimensional (2D) islands laying flat on the support], the ratio is roughly constant, but if the Pd deposits as multilayer structures on the surface (clusters with 3D structure), then the ratio decreases because only the top-layer Pd is detected. From comparison with previous data for Ni_n/TiO₂ and Ir_n/TiO₂, we believe that Pd₂ to Pd₁₀ clusters deposit as single-layer islands (isolated clusters, flat on the surface), whereas Pd₁₆ through Pd₂₅ show signs that a second layer is beginning to appear (isolated clusters with some 3D structure). Ni_n/TiO₂(110) also shows the transition to two-layer clusters above Ni₁₀, as might

be expected for metals in the same group (22). Comparing the ISS and CO oxidation activity results might suggest that the onset of second-layer formation could be correlated with increased activity for Pd₁₆ and Pd₂₀. However, Pd₂₅ continued the trend toward increasing second-layer filling, but its reactivity was low. Furthermore, the ISS-reactivity correlation did not exist for the smaller clusters, in which non-monotonic reactivity was observed, and ISS remained essentially constant.

The anomalously small ISS ratio for Pd₁/TiO₂ is similar to what is observed for Ni and Ir atoms on TiO₂, and the origin of the effect is unclear. It may be an electronic effect of atom interactions with the surface or some effect of atomic binding geometry. We can rule out agglomeration of atoms into clusters as a possible explanation because

if clusters formed, we would expect them to be highly active, in contrast to their apparent total inertness (Fig. 2).

The remaining question is what the strong correlation between activity and Pd 3d BE tells us about the chemistry. The core electrons are not involved in bonding, but they indirectly reflect the valence electronic structure, which is the controlling factor. We are interested in the chemical, or initial state contribution to the binding-energy shifts, and to get this we must correct for final state effects. A procedure based on shifts in Auger lines has been suggested for estimating the final state effect (32, 33); however, Holneicher *et al.* (34) pointed out that this method is invalid for metals like Pd, in which the available Auger transitions involve valence levels. To separate the initial and final state effects quantitatively will probably require detailed theory, but the chemistry itself provides an indication of the importance of initial state contributions. Low reactivity suggests that samples like Pd₁/TiO₂ and Pd₇/TiO₂ (and in context Pd₂₅/TiO₂) have particularly stable valence structures. A stable valence shell would also tend to increase core-level BE both because the initial state is more stable and because a stable valence shell would tend to be less polarizable, reducing final state stabilization.

One surprise is that the BE for Pd₂₀/TiO₂ is at the limit for bulk Pd. Given that ISS shows just the beginning of a second layer for our largest clusters, Pd is not really in a bulk-like environment. Furthermore, the BE goes back up substantially for Pd₂₅. This pattern reflects cluster size-dependent initial and final state effects that just happen to cancel each other for Pd₂₀, resulting in a value near the bulk limit. Final state effects (charge localization and reduced screening as compared with bulk Pd) tend to shift the BEs to higher energy. To cancel this shift, there must be an initial state shift to lower BE, such as that from a net transfer of electron density from the TiO₂ support to the Pd clusters. Of course, electron transfer to the clusters will also tend to have substantial effects on their ability to activate O₂, which is presumably the limiting step in the CO oxidation reaction.

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Fig. S1

References

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Dynamical Steering and Electronic Excitation in NO Scattering from a Gold Surface

Neil Shenvi,* Sharani Roy,*† John C. Tully‡

Nonadiabatic coupling of nuclear motion to electronic excitations at metal surfaces is believed to influence a host of important chemical processes and has generated a great deal of experimental and theoretical interest. We applied a recently developed theoretical framework to examine the nature and importance of nonadiabatic behavior in a system that has been extensively studied experimentally: the scattering of vibrationally excited nitric oxide molecules from a Au(111) surface. We conclude that the nonadiabatic transition rate depends strongly on both the N-O internuclear separation and the molecular orientation and, furthermore, that molecule-surface forces can steer the molecule into strong-coupling configurations. This mechanism elucidates key features of the experiments and provides several testable predictions regarding the dependence of vibrational energy transfer on the initial vibrational energy, molecular orientation, and incident angle.

The pathways of atomic motions during a chemical event on a metal surface do not follow the same rules that govern most gas-phase or liquid-phase chemical reactions. Because metals exhibit a continuous spectrum of infinitesimally spaced electronic states, the Born-Oppenheimer approximation (1)—the cornerstone of chemical reaction theory—is suspect. There is ample evidence of dramatic departures from adiabatic (Born-Oppenheimer) behavior, that is, of electronic excitation induced by atomic motion (2). Experimental demonstrations include dramatic shortening of adsorbate molecule vibrational lifetimes (3), the detection of electric currents (“chemi-currents”) promoted by chemical reaction (4–7), and the emission of light from excited electronic states (8). Particularly detailed and compelling evidence has emerged from recent experiments by Wodtke and coworkers on the scattering of

vibrationally excited nitric oxide molecules from the (111) face of a gold (Au) crystal. These experiments have revealed a number of fascinating features, including a high probability of multi-quantum vibrational transitions (9, 10), trapping (sticking) probabilities independent of the initial NO vibrational state (11), an inverse correlation between the vibrational energy lost by a scattered NO molecule and its final rotational energy (12), electron emission from a low-work function cesium (Cs)-covered Au surface promoted by NO vibration (13, 14), and inverse dependence of the electron emission probability on the incident molecule velocity (15). We present here a comprehensive picture that successfully encompasses and elucidates all of these observations. As we show below, the reorientation of the molecule into regions of strong nonadiabatic coupling—dynamical steering—plays an important role in the process.

There are two basic components of our theoretical strategy: (i) calculation of the multiple potential energy surfaces (PESs) that arise from the interaction of a NO molecule with a Au(111) surface (16, 17) and (ii) simulation of non-adiabatic molecule-surface scattering subject to this myriad of PESs (17). In order to make the

first task tractable, we assumed that although large numbers of electronic states must be included to allow for multiple electron-hole-pair (EHP) excitations in the metal, the NO molecule can exist in only two local electronic configurations, neutral molecule and negative ion (16). This treatment embodies the major electronic coupling mechanism proposed by Wodtke and coworkers (9, 10, 18): the transfer of an electron from an occupied level of the metal onto the molecule to form the negative ion and subsequent electron transfer back to an unoccupied metal state. Beginning with Gadzuk (19), this electron transfer mechanism has been widely invoked in the field of gas-surface dynamics, including previous theoretical studies of NO scattering from metals (20, 21). With this simplifying assumption, we previously computed neutral and negative ion PESs for NO/Au(111) using density functional theory (DFT), with and without application of a weak electric field to untangle neutral and negative ion contributions. Details are reported elsewhere (16). The difference in energy between the neutral and ionic PESs defines the affinity level, that is, the energy of the electron acceptor orbital of the molecule.

Next, we approximated the metallic continuum by M discrete levels which, with the addition of the affinity level of the molecule, produces an $(M+1)$ by $(M+1)$ independent-electron Newns-Anderson Hamiltonian matrix. Each element of the matrix is a function of the positions of all of the atoms, fit to convenient analytic expressions, as described previously (17). The eigenvalues and eigenvectors of the matrix are one-electron energies and orbitals, with each orbital a linear combination of the M discretized metallic states and the single localized NO affinity level. An adiabatic N -electron wave function is then a single Slater determinant of occupied one-electron states. A huge number of adiabatic N -electron states can result from this procedure; for $N = 20$ and $M = 40$, the values we used for the present application, there are more than 10^{11} adiabatic states, that is, permutations of 20 electrons in 41 orbitals. By testing convergence with larger values of M , we verified that $M = 40$ provides sufficient low-

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energy excitations to incorporate electronic friction, the loss of nuclear kinetic energy via EHP excitations (22), and includes electronic splittings comparable to the ~ 0.2 eV splittings between NO vibrational levels.

The ground state of the Newns-Anderson Hamiltonian, obtained by filling the 20 lowest-energy orbitals, accurately reproduces the DFT ground-state energy and local charge at geometries for which the latter were deemed reliable. Figure 1A is a contour plot of the fractional negative charge on the NO molecule in the ground state, fit to DFT results, when the NO bond axis is perpendicular to a threefold site on the frozen Au(111) surface, N atom down. The strong variation of the charge with N-O internuclear separation, r , supports the vibrationally mediated charge-transfer picture proposed by Huang *et al.* (9). The rapidly oscillating curve in Fig. 1A is the incoming leg of a sample trajectory for a vibrationally excited ($v = 15$) NO molecule. The high-frequency vibrational motion of the molecule promotes multiple rapid excursions into the high-negative-charge region, enhancing nonadiabatic behavior. Figure 1B is analogous to Fig. 1A, but with the molecule oriented with the O end toward the surface. For this orientation, the trajectory barely enters the region of substantial negative-ion character, in contrast to the behavior when the N atom points toward the surface. This result offers a first glimpse of the orientational steering mechanism that we believe underlies many of the experimental observations.

The second component of our strategy is realistic simulation of the nonadiabatic scattering of the NO molecule from the Au(111) surface, as governed by the enormous numbers of electronic PESs encompassed in the Newns-Anderson Hamiltonian. We applied a variation of surface hopping (23), a stochastic procedure in which trajectories move on a single adiabatic PES, subject to instantaneous transitions to different PESs, with hopping probabilities governed by the evolving amplitudes of each electronic state. This task appears daunting in view of the more than 10^{11} adiabatic states among which to hop in this application. However, it is actually quite feasible because each one-electron orbital of the single determinant wave function evolves independently. Surface hops are one-electron events as well, simply requiring the replacement of a single orbital in the Slater determinant. The formulation of this independent electron surface hopping (IESH) method was described previously (17). Here, we report the application of this method to elucidate the factors that underlie vibrationally inelastic scattering of NO from Au.

The first of the five experimental features listed above is the high probability of multiquantum vibrational transitions during a direct scattering event. We see relatively good agreement between the experimental final vibrational energy distribution for scattering of a NO ($v = 15$) molecule from Au(111) (Fig. 2A) and the results of the nonadiabatic IESH simulations (Fig. 2D). Also shown

are the results of adiabatic (ground electronic state) simulations (Fig. 2C), which compare better with experimental scattering of NO ($v = 12$) from an insulating LiF(001) surface (Fig. 2B), supporting the conclusion that nonadiabaticity dominates vibrational energy transfer for NO/Au(111) scattering.

The second experimental feature, the independence of the trapping probability on initial vibrational energy, was also reproduced with the IESH simulations for the cases studied experimentally, $v = 0$ versus $v = 2$. Adiabatic simulations (Fig. 3A) predict that the trapping probability increases with increasing vibrational energy, which is a con-

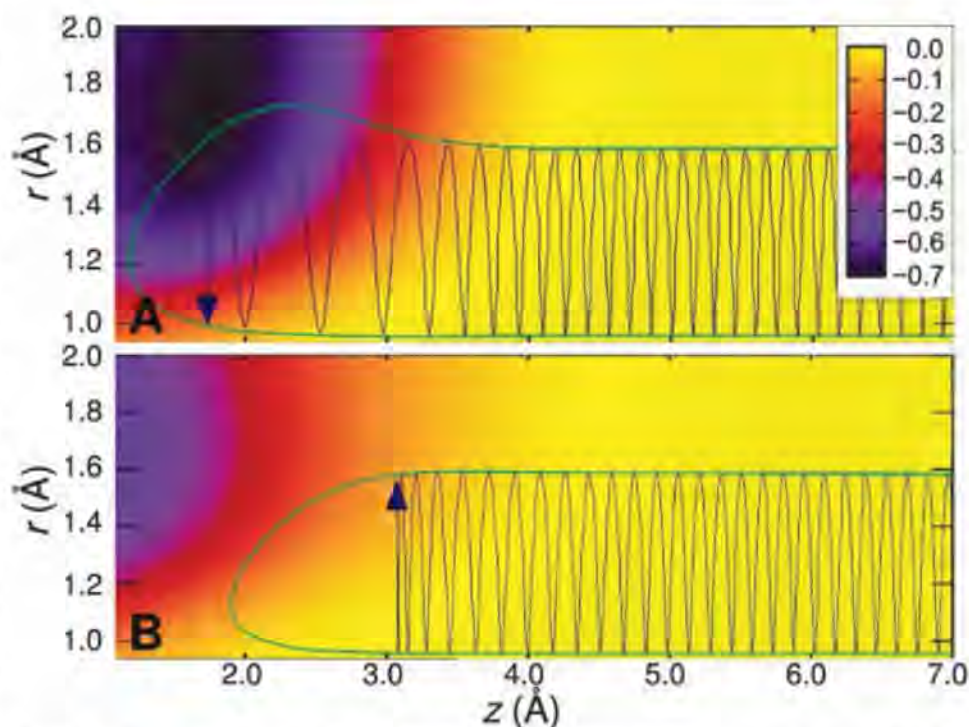
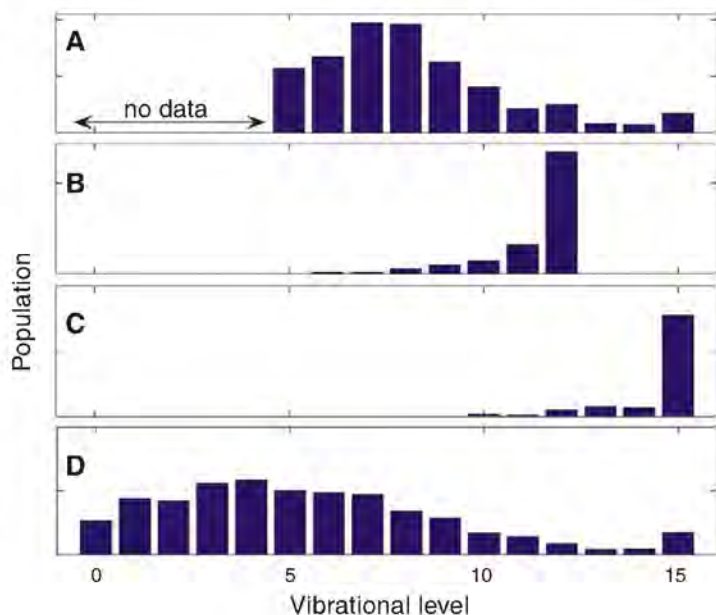


Fig. 1. The rapidly oscillating curves are the incoming legs of two sample trajectories for NO scattering from a perfectly crystalline Au surface at normal incidence above a threefold hollow site, with initial translational energy of 5.3 kJ/mol, initial vibrational energy corresponding to $v = 15$, and the molecular axis oriented along the surface normal. z is the distance of the center of mass of the molecule from the surface; r is the N-O internuclear separation. (A) The molecule is oriented with the N end toward the surface. (B) The molecule is oriented with the O end toward the surface. The background raster plots show the partial negative charge on the NO molecule obtained from a fit to ground-state DFT results. The green curves demarcate the regions classically accessible to a NO molecule in the $v = 15$ state with zero initial translational and rotational energy.

Fig. 2. The final vibrational state distribution for NO scattered from a room-temperature surface at normal incidence with initial translational energy of 4.8 kJ/mol. (A) Experiments for a Au(111) surface and NO ($v = 15$), from (9). (B) Experiments for an insulating LiF(001) surface and NO ($v = 12$), from (24). (C) Adiabatic molecular dynamics simulations for a Au(111) surface and NO ($v = 15$). (D) Nonadiabatic IESH simulations for a Au(111) surface and NO ($v = 15$).



sequence of the average binding energy of NO to the surface increasing with increasing bond length because of an increase of its ionic character. Nonadiabatic transitions lower the trapping probability (Fig. 3B) relative to the adiabatic result by promoting the transfer of vibrational energy into electronic excitation as well as into translational motion via the adjustment of nuclear velocities along the nonadiabatic coupling vector. For $v = 15$ (not measured), the IESH simulations predict that the trapping probability will be very low because of the highly nonadiabatic behavior of highly excited vibrational states. This low trapping probability for highly vibrationally excited molecules awaits experimental verification.

The third experimental feature is addressed in Fig. 3C, which shows the mean rotational energy for scattering of NO ($v = 2$) from Au(111) as a function of initial translational energy, plotted separately for final NO vibrational states $v = 2$ and $v = 1$. Both the experiment (12) and IESH show an inverse correlation; the deposited vibrational energy does not increase the rotational energy. Rather, the rotational energy is lower, even though there is more energy available. Our simulations reveal that this effect is, in part, a consequence of dynamical steering; upon approach, the NO molecule experiences a torque that steers the molecule toward its most favorable orientation, with the N end of the molecule pointing to the surface.

Fig. 3. (A) Trapping probability of NO on the Au(111) surface as a function of incidence energy and initial vibrational state. Blue, green, and red lines denote the results of adiabatic molecular dynamics simulations for the $v = 0$, $v = 2$, and $v = 15$ states, respectively. Blue Xs and green circles indicate experimental trapping probability measurements for $v = 0$ and $v = 2$, respectively, from (11). (B) The same trapping probabilities calculated by using nonadiabatic IESH dynamics, compared with the same experimental results. (C) Mean rotational energy for scattered molecules incident in $v = 2$ that undergo elastic (green) and inelastic (magenta) scattering as a function of incident energy. Lines are results of IESH simulations, and markers indicate experimental values from (12). (D) The energy distribution of excited conduction electrons in the metal after scattering of NO ($v = 15$). The IESH results (solid curve) predict a much higher probability of high-energy electrons than the friction model (dashed curve). Error bars on calculated results indicate 1 SD.

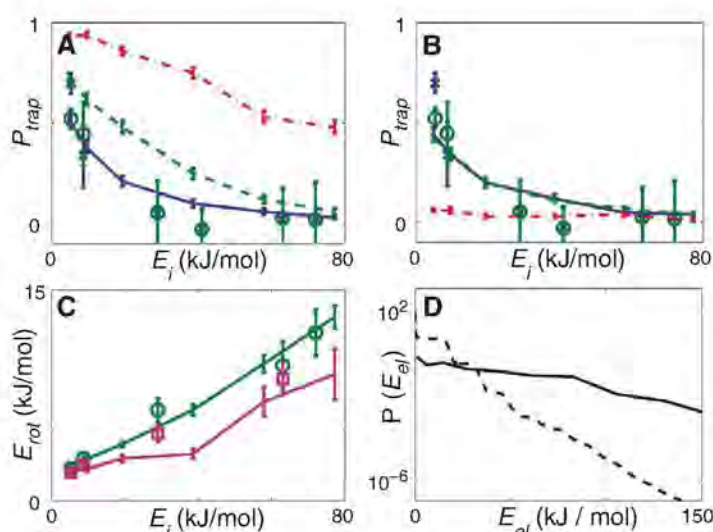
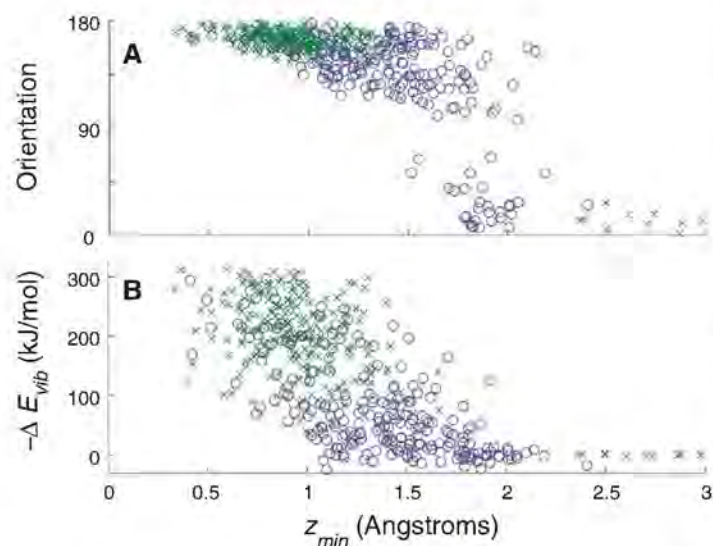


Fig. 4. (A) The correlation between the minimum atom-surface distance achieved during a trajectory and the molecular orientation at that minimum. Each point represents a single IESH trajectory for NO ($v = 15$). Circles represent an incidence energy of 77 kJ/mol and Xs represent an incidence energy of 4.8 kJ/mol. Only trajectories that are strongly oriented with the N atom pointing to the surface can achieve a close approach. (B) The correlation between the minimum molecule-surface distance achieved and the extent of vibrational energy loss. Trajectories that achieve a close approach to the surface through dynamical steering are more likely to lose large amounts of vibrational energy. At higher incidence energies, vibrational relaxation decreases because dynamical steering is less effective.



Nonadiabatic vibrational energy transfer is most efficient in this geometry, as shown in Fig. 1. Molecules that approach the surface with approximately this orientation tend to experience the smallest torque and therefore less rotational excitation. Figure 4A shows the strong correlation of the distance of closest approach of the NO molecule with the orientation of the NO axis at the closest approach position. Surprisingly, faster (77 kJ/mol) molecules do not necessarily penetrate closer to the surface than slower (4.8 kJ/mol) molecules; slower molecules have more time to achieve the favorable, N-atom-down orientation required to get close to the surface. Figure 4B shows the strong correlation between vibrational energy lost by NO ($v = 15$) and its distance of closest approach. Fig. 4 offers compelling support for the importance of dynamical reorientation on nonadiabatic energy transfer. The small elastic peak at $v = 15$ that was observed both experimentally and theoretically (Fig. 2, A and D) can now be understood as the signature of trajectories that strike with the O end toward the surface.

Emission of an electron from the cesiated Au surface (13, 14), the fourth experimental feature, requires that at least 150 kJ/mol of energy be deposited into a single electron to overcome the work function. This corresponds to at least 8 quanta of NO vibration, a multiquantum event. We have not examined the Cs-covered surface, and certainly the presence of Cs atoms will do more than simply lower the work function of the Au surface. Nevertheless, it is instructive to examine our calculated electron energy distribution for NO scattering from the clean Au surface. As shown in Fig. 3D, the IESH simulations do predict a substantial population of electrons with energies up to 150 kJ/mol, in contrast to the electronic friction theory (22) applied with the same NO-Au interaction parameters.

The final experimental puzzle is the observed decrease of the electron emission yield from Cs-Au with increasing velocity of the incident vibrationally excited NO (15). As noted by Nahler *et al.*, this observation appears counterintuitive; nonadiabatic behavior is expected to be more important at higher velocities. Although a direct comparison of our simulations on clean Au(111) to the experiments on cesiated Au(111) is not possible, it is intriguing that we observed the same trend, in which the mean energy deposited into electronic excitation decreases with increasing incident velocity. Because nonadiabatic transitions are driven primarily by vibrational motion in this system, the increased probability of entering the strong coupling region dominates the direct influence of lower translational energy on the nonadiabatic transition probability. Our proposed dynamical steering mechanism leads to experimentally testable predictions about the dependence of vibrational energy loss on increasing incident velocity (lower), incident angle (lower at grazing angles), and increasing incident rotational energy (lower). Perhaps most dramatically, we predict much larger vibrational energy loss for

oriented NO beams in which the molecules are aligned to favor N-end approach of the molecule to the surface. It is our hope that these predictions will motivate new experiments to test the validity of our mechanism and ultimately to enhance our understanding of nonadiabatic processes at metal surfaces.

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Chemically Accurate Simulation of a Prototypical Surface Reaction: H₂ Dissociation on Cu(111)

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Methods for accurately computing the interaction of molecules with metal surfaces are critical to understanding and thereby improving heterogeneous catalysis. We introduce an implementation of the specific reaction parameter (SRP) approach to density functional theory (DFT) that carries the method forward from a semiquantitative to a quantitative description of the molecule-surface interaction. Dynamics calculations on reactive scattering of hydrogen from the copper (111) surface using an SRP-DFT potential energy surface reproduce data on the dissociative adsorption probability as a function of incidence energy and reactant state and data on rotationally inelastic scattering with chemical accuracy (within ~4.2 kilojoules per mole).

Reactions of gas- or liquid-phase molecules with metal surfaces are of tremendous practical importance, because the production of most synthetic compounds involves heterogeneous catalysis in such conditions. One of the achievements recognized by the 2007 Nobel Prize in Chemistry, awarded to G. Ertl, was the detailed description of the sequence of elementary molecule-surface reactions by which vast quantities of ammonia are produced for fertilizer (1).

A major difficulty in modeling molecule-metal surface reactions is that reaction barriers for these systems cannot yet be calculated with chemical accuracy (1 kcal/mol ≈ 4.2 kJ/mol), even for the system's ground electronic state.

The best electronic structure method available for these reactions since 1994 (2, 3) is density functional theory (DFT) with functionals incorporating a generalized gradient approximation (GGA) or a meta-GGA. Systematic investigations of gas-phase reactions, which are not yet available for molecule-surface reactions, show that barriers calculated with GGAs or meta-GGAs for the former reactions exhibit mean absolute errors (MAEs) ≥ 3 kcal/mol (4). In contrast, very high accuracy (MAEs < 1 kcal/mol) is available for gas-phase reactions from either high-level ab initio theory (4, 5) or DFT with hybrid functionals (4). High-level ab initio methods and hybrid DFT are currently too computationally intensive to map out a potential energy surface (PES) for a molecule-surface reaction. Even though hybrid DFT can now be used to compute a few points of such a PES (6), fundamental problems hamper a simultaneously accurate description of the molecule and the metal surface with this method (7, 8). The lack of chemical accuracy for ground state molecule-metal surface systems stands in the way of a quantitative description of heterogeneously catalyzed reactions (9) and many reactions involving excited electronic states (10–12).

Methods for deriving molecule-surface interaction energies are best tested through compar-

isons with experiments for systems that are not substantially affected by electron-hole pair excitations and surface vibrations (phonons). In H₂-metal systems, reaction and diffractive scattering can be accurately described with the Born-Oppenheimer approximation (13, 14). Previous research has suggested that phonons have only a small effect on the reaction of H₂ on metal surfaces (15, 16). Quantum dynamical calculations on scattering of H₂ from metal surfaces using the electronically adiabatic, static surface model with explicit treatment of all six molecular degrees of freedom of H₂ have already allowed detailed comparisons with experiments (15, 16). These features make H₂-metal surface systems ideal for testing ideas and for evaluating the state-of-the-art in electronic structure theory for molecule-surface reactions.

Among the H₂-metal systems, H₂ + Cu(111) is an excellent prototype system. Information on the influence of the collision energy E_i and the molecule's initial vibrational state v on the probability of dissociative adsorption [H₂(g) → 2H(ad)] is provided by molecular beam experiments (17, 18). Associative desorption experiments yield additional information on the influence of the molecule's initial rotational state j (17, 18) and its orientation on reaction (19). The validity of using detailed balance to obtain information on the dissociative adsorption reaction from experiments on the associative desorption reaction has been established (17, 18). Experiments also provide information on rotationally (20) and vibrationally (21, 22) inelastic scattering of H₂ from Cu(111).

So far, comparisons of these experiments with calculations modeling motion in all six molecular degrees of freedom have been limited in scope. Previous six-dimensional dynamical calculations used PESs based on DFT-GGA calculations (23–25). The GGA PESs used in these calculations gave qualitatively wrong results for vibrationally inelastic scattering [vibrational excitation probabilities <1% versus experimental values >20% (22)] and allowed only a semiquantitative description of reaction (16).

Here, we successfully advance from a semiquantitative to a quantitatively accurate descrip-

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tion of the reactive interaction of a molecule with a metal surface, using an implementation of the specific reaction parameter (SRP) approach to DFT adapted to molecule-metal surface systems (26). We present dynamics calculations on the H_2 and $D_2 + Cu(111)$ system using an SRP-DFT PES and an electronically adiabatic static surface model. Excellent accuracy is achieved for those experiments for which the dynamical model is expected to hold, either because there is not much excitation of the surface degrees of freedom, or

because the experimental results can be extrapolated to low surface temperature (T_s) using existing information. Specifically, our SRP-DFT results for H_2 agree with experiment to within chemical accuracy for the overall reaction probability measured with molecular beams, for the dependence of the reaction probability on the molecule's initial vibrational and rotational state, and for rotationally inelastic scattering.

Our dynamical calculations treat the motion of H_2 (D_2) in all six degrees of freedom (27). To obtain an accurate PES, we modified the SRP DFT approach as first applied to gas-phase reactions (26) to allow the computation of molecule-surface interaction energies. In our implementation, the exchange-correlation functional is a weighted average of two GGAs. We adopt the premise that one functional should underestimate and one should overestimate the reactivity. Experiments and theoretical studies on another H_2 -metal surface reaction [the $H_2 + Ru(0001)$ system (28)] and research on ammonia production (9) guided us to choose the SRP exchange correlation functional $E_{XC}^{SRP} = xE_{XC}^{RPBE} + (1-x)E_{XC}^{PW91}$, combining the PW91 (29) and RPBE (30) functionals. The rationale underlying the SRP-DFT method is that by fitting the parameter x in E_{XC}^{SRP} to one suitable experiment, a functional can be obtained that will yield a PES

that not only contains the correct barrier height(s) but also enables an accurate description of other (more detailed) dynamics experiments (27).

The SRP functional was determined (27) by adjusting x to best describe the reaction probabilities for $D_2 + Cu(111)$ from molecular beam experiments that used a low surface temperature ($T_s = 120$ K) and achieved high E_i by seeding D_2 with faster traveling H_2 (18). Reaction probabilities were computed with both a PW91 and an RPBE PES (Fig. 1A): PW91 overestimates and RPBE underestimates the observed reaction probabilities. The use of the SRP-DFT PES obtained with $x = 0.43$ (figs. S1 and S2 and table S3) in quasi-classical dynamics calculations yields reaction probabilities that agree with experiment to within chemical accuracy (4.2 kJ/mol): The collision energy spacing between the experimental data and the cubic spline interpolated reaction probability curves is less than or equal to 4.2 kJ/mol (Fig. 1A). Because the reaction probability reflects the proportion of impact sites and molecular orientations for which the collision energy exceeds the barrier height (27), this signifies that the SRP-DFT approach describes the minimum barrier height and the variation of the barrier height with impact site and orientation with chemical accuracy.

Reaction probabilities computed for $D_2 + Cu(111)$ are also in excellent agreement with experiments that used pure D_2 beams (18) (fig. S8). Likewise, reaction probabilities computed for $H_2 + Cu(111)$ are in excellent agreement with two rather different sets of experimental data for pure H_2 beams (17, 31) (Fig. 1B). The calculations thereby present the solution to a previously unsolved puzzle (17): At similar E_i , these two sets of reaction probabilities differed by an order of magnitude. Analysis of the beams (fig. S4 and supporting text) used in both experiments shows that the two sets of reaction probabilities are consistent; the reaction probabilities of Berger *et al.* were larger because those authors used beams with wider velocity distributions, the tails of which overlap to a greater extent with the portion of the energy-resolved reaction probability curve that steeply rises above threshold (fig. S5). (The "collision energy" in Fig. 1, A and B, is in fact the average collision energy.) Our findings suggest that earlier comparisons of theory to experiment for H_2 reactions on metal surfaces should be reevaluated by including energy convolution effects.

Information on how changing the initial rotational and vibrational state of H_2 affects its reactivity is contained in the dependence of the initial rovibrational state-resolved reaction probability $R_{vj}(E_i)$ on v and j . Computed $R_{vj}(E_i)$ values agree with results extracted from experiments for H_2 (17) and D_2 (18, 32) to within a small energy shift for reaction probabilities up to about 0.75 times the maximum experimental value (Fig. 1C and fig. S9). We can therefore assess the quality of the theory by considering the size of the shifts that, when applied to the

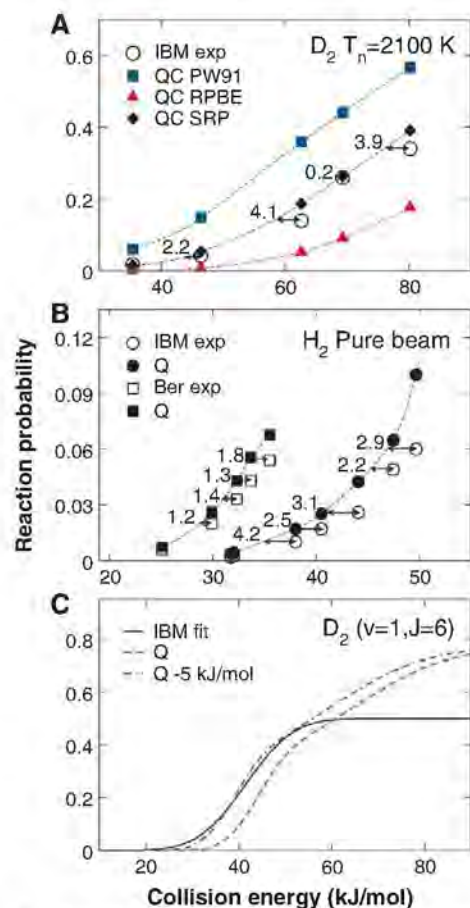


Fig. 1. Reaction probabilities computed with the quasi-classical trajectory (QC) method and with quantum dynamics (Q) using SRP-DFT are compared with experimentally measured values for D_2 (18) and H_2 (17, 31) + $Cu(111)$. Experimental results from (17, 18) are labeled "IBM" and those from (31) "Ber". (A) Comparison to experiments on D_2 using a nozzle temperature of 2100 K. Reaction probabilities computed with the PW91 and the RPBE PES are also shown. The QC results exhibit statistical errors, but in all cases the SDs are ≤ 0.005 . (B) Comparison to two experiments using pure H_2 beams with different velocity distributions (17, 31) [see also (27)]. (C) The $R_{vj}(E_i)$ extracted from experiments (18, 32) is compared to the $R_{vj}(E_i)$ computed with quantum dynamics, and to the computed $R_{vj}(E_i)$ curve shifted down by -5 kJ/mol, for $(v=1, j=6)$ D_2 . In (A) and (B), the arrows and the accompanying numbers (in kJ/mol) show the collision energy spacing between the experimental data and the cubic spline interpolated SRP-DFT reaction probability curves.

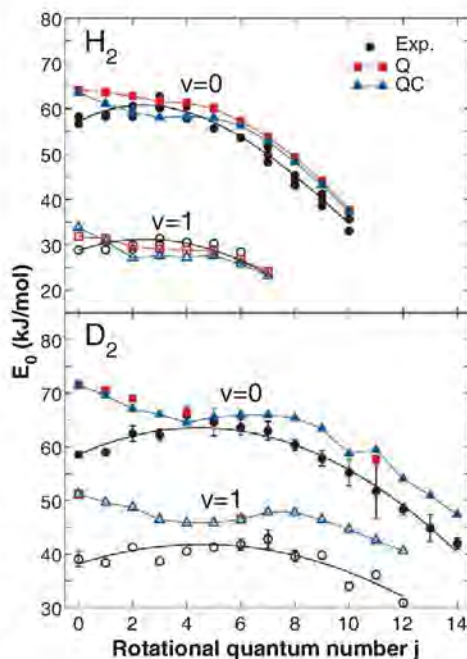
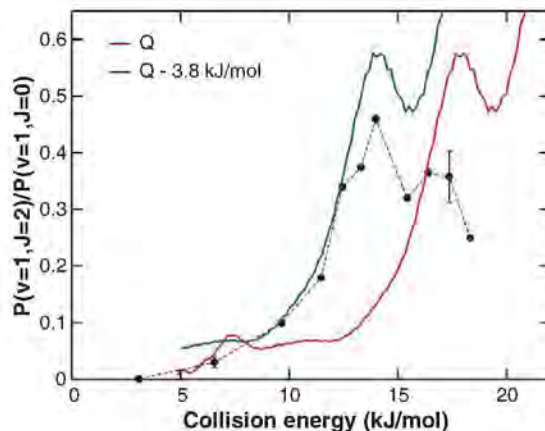


Fig. 2. Effective barrier heights $E_0(v,j)$ computed with the QC method and with quantum dynamics (Q) are compared with values extracted from experiments (27) for H_2 (17) and D_2 (18, 32) + $Cu(111)$. For H_2 , raw extracted experimental $E_0(v,j)$ values are shown for repeated measurements (17). For D_2 , raw extracted experimental $E_0(v,j)$ values are presented with error bars representing 66% confidence intervals, where available (18, 32). The black curves represent polynomial fits of the experimental data (17, 32).

Fig. 3. Comparison of theoretical and experimental ratios of rotationally inelastic scattering probabilities $P(v=1, j=0 \rightarrow v=1, j=2)/P(v=1, j=0 \rightarrow v=1, j=0)$ for H_2 colliding with Cu(111). Experimental data are from (20). The error bars represent maximum deviations in repeated measurements and are estimates of the SDs (40). Theoretical values were computed with SRP-DFT quantum dynamics and are also shifted down in energy by 3.8 kJ/mol.



theoretical $R_{vj}(E_i)$ curves, effectively superimpose these curves on the experimental $R_{vj}(E_i)$ curves. These shifts are simply taken as the differences between the theoretical and experimental values of the effective barrier heights $E_0(v, j)$, $E_0(v, j)$ being the collision energy at which the reaction probability first becomes equal to half the fitted maximum experimental value (27). The good agreement between theory and experiment for the $E_0(v, j)$ values (Fig. 2) then shows that theoretical and experimental $R_{vj}(E_i)$ curves of H_2 agree to within chemical accuracy for $v=0$, $j>0$ and for $v=1$, the MAE in $E_0(v, j)$ being 2.5 kJ/mol. For D_2 , the agreement obtained with quasi-classical dynamics (the quality of which is confirmed by direct comparison to quantum dynamics for H_2 in Fig. 2) is also good. Chemical accuracy is achieved for ($v=0, j=3$ to 7 and $j=10$) and for ($v=1, j=3$ to 4), and the MAE in $E_0(v, j)$ is 6.2 kJ/mol (Fig. 2). The above comparison between theory and experiment was based on polynomial fits the experimentalists made of their extracted $E_0(v, j)$ values (17, 32), but similar conclusions are reached if the comparison is based on the $E_0(v, j)$ values directly extracted from the experiments (17, 18, 27, 32) (Fig. 2).

For rotationally inelastic scattering, computed ratios of probabilities $P(v=1, j=0 \rightarrow v=1, j=2)$ and $P(v=1, j=0 \rightarrow v=1, j=0)$ are in very good agreement with experiments performed at $T_s = 300$ K (20) (Fig. 3). Shifting the theoretical results by -3.8 kJ/mol (33) almost superimposes the theoretical on the experimental data up to about $E_i = 14$ kJ/mol, i.e., the computed and experimental rotational excitation probability curves agree to within chemical accuracy (Fig. 3). Previously, quantum dynamics calculations greatly overestimated rotational excitation probabilities for $H_2 + Cu(100)$ (34). Because the barriers in the PESs used were too low, the incident H_2 could access regions of the PES where a strong orientational dependence of the H_2 -surface interaction promotes rotational excitation. At $E_i \geq 14$ kJ/mol, the theory for $H_2 + Cu(111)$ starts to differ from experiment, but the experimentalists note that for these energies the ratio becomes more difficult to measure.

We also performed calculations on the effect of molecular orientation on reaction of D_2 and on vibrational excitation of H_2 . For these calculations, the level of agreement with experiment was not as high as reported for the experiments considered above. We attribute this lack of agreement to neglect of the surface degrees of freedom in our dynamical model (27). Additionally, it is unclear how the influence of H_2 orientation on reaction and how vibrational excitation at the high T_s prevailing in experiments can be extrapolated to low T_s , for comparison to our electronically adiabatic, static surface model calculations (27). Finally, an accurate simulation of experimental time-of-flight spectra exhibiting vibrational excitation (22) requires information (27) on how much energy is lost to the surface upon vibrationally inelastic scattering (35), which is absent for vibrational excitation of H_2 scattering from Cu(111).

The SRP-DFT method can be extended to reaction of heavier molecules, where even at low T_s , phonon effects are important (36). In such applications, the dynamics calculations should incorporate phonons at the stage when the SRP functional is fit to experimental data. Approximate ways of including the effect of phonons can be incorporated in quantum dynamical (36) and ab initio molecular dynamics (37) simulations. It may even be possible to extend SRP-DFT to improve the description of scattering involving excited states, for instance, by adjusting the SRP functional for each spin (12) or charge (38) state of the molecule involved. Finally, whereas the SRP-DFT procedure used here is semi-empirical (i.e., fit to experimental data), applications may be envisaged in which the SRP functional is parameterized to reproduce a limited set of molecule-surface interaction energy data obtained with a computationally intensive electronic structure method with a claim to high accuracy (39).

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Supporting Online Material

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Materials and Methods

SOM Text

Figs. S1 to S9

Tables S1 to S11

References and Notes

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Shifts in Lake N:P Stoichiometry and Nutrient Limitation Driven by Atmospheric Nitrogen Deposition

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Human activities have more than doubled the amount of nitrogen (N) circulating in the biosphere. One major pathway of this anthropogenic N input into ecosystems has been increased regional deposition from the atmosphere. Here we show that atmospheric N deposition increased the stoichiometric ratio of N and phosphorus (P) in lakes in Norway, Sweden, and Colorado, United States, and, as a result, patterns of ecological nutrient limitation were shifted. Under low N deposition, phytoplankton growth is generally N-limited; however, in high-N deposition lakes, phytoplankton growth is consistently P-limited. Continued anthropogenic amplification of the global N cycle will further alter ecological processes, such as biogeochemical cycling, trophic dynamics, and biological diversity, in the world's lakes, even in lakes far from direct human disturbance.

All of Earth's major biogeochemical cycles have been altered by human population expansion and industrialization (1). In particular, the total amount of circulating nitrogen (N) in the biosphere has increased by more than 100% (2). Much of this N is in the form of oxidized and reduced reactive N species (NO_x and NH_x), which are produced as byproducts of fossil fuel combustion and from agricultural emissions from croplands, rangelands, and industrial livestock feeding operations. Reactive N is transported regionally through the atmosphere and deposited in terrestrial and aquatic habitats via rain, snowfall, and dry deposition (3). Considerable previous work has evaluated the biogeochemical and ecological impacts of this atmospheric N deposition on terrestrial ecosystems (4) and was performed because primary production in terrestrial systems is often limited by N availability (5). The effects of atmospheric N deposition on freshwater ecosystems, however, have not been widely studied, perhaps because lake primary production is generally thought to be limited by phosphorus (P) (6). However, the purported primacy of P limitation of lake productivity (7) has been challenged by some recent experimental and comparative assessments, suggesting frequent phytoplankton N- and light-limitation in lakes (8, 9). The effects of atmospheric N deposition on lake phytoplankton have several important implications. For example, fundamental phytoplankton biomass-P loading relationships may be a function of atmospheric N inputs (10). Furthermore, increased N:P supply ratios might reduce phytoplankton diversity by favoring those relatively few species with strong

competitive abilities for using P (11). Enhanced phytoplankton P limitation caused by elevated N loading from the atmosphere may also affect the functioning of lake food webs, because P-limited algae are known to be poor-quality food for consumers such as zooplankton (12).

We analyzed 2053 lakes in Norway (385 lakes) and Sweden (1668 lakes) that represent both high- and low-N deposition conditions to determine whether elevated atmospheric N inputs affect lake phytoplankton nutrient supplies in terms of concentrations and ratios of total N (TN) and total P (TP). Additionally, we performed a comprehensive study of nutrient concentrations and phytoplankton nutrient limitation (via N and P enrichment bioassay experiments) in high- and low-deposition lakes both in southern Norway and in the central Colorado Rocky Mountains, United States (13). These data were combined with data from previous studies on high- and low-deposition lakes in southern and northern Sweden (14), resulting in a overall assessment involving nearly 90 N:P enrichment experiments.

In each of the three study regions, lakes receiving elevated N deposition had significantly elevated surface-water nitrate (NO_3^-) concentrations relative to low-deposition lakes (~sevenfold higher overall, Table 1). Increased N deposition was also associated with considerably higher overall lake N concentrations and higher availability of N compared to P (Table 1 and table S1). In the lakes sampled for the bioassay studies (Table 1), average TN:TP ratios were about 2 to 5 times higher in high-deposition lakes. This pattern was confirmed in the large Scandinavian data set, in which the slopes of TN versus TP relationships in Norway and Sweden were 2 to 2.5 times higher in high-deposition lakes than in lakes receiving N loading closer to background levels (Fig. 1). The slopes of the TN versus TP relationships in low-deposition lakes in the large-scale Norway and Sweden lake surveys (Fig. 1) were similar to the average TN:TP ratios observed in the lakes used in our experimental studies in Norway and Sweden (Table 1), suggesting that the low-deposition lakes involved in our bioassays were representative of the larger population of

unaffected Scandinavian lakes. However, TN:TP ratios in the Scandinavian high-N deposition lakes included in the experimental studies (Table 1) were somewhat higher than the slopes of the TN versus TP relationships, perhaps indicating that our experimental studies involved lakes with stronger N deposition than the overall population classified as "high deposition" in the lake survey analyses.

Changes of N:P stoichiometry indicate altered patterns of phytoplankton nutrient limitation. Nutrient enrichment bioassays demonstrated an inverse relationship between phytoplankton N and P limitation, consistent with Liebig-type resource limitation (15) in which significant changes are observed in response to one nutrient or the other (or neither), but not to enrichments of N or P made separately in the same experiment [Fig. 2A; lakes with high values of both response ratios (defined as the final chlorophyll levels normalized to control samples) for N (RR-N) and for P (RR-P) were not observed]. The data indicate shifts in phytoplankton nutrient limitation caused by elevated atmospheric N deposition in each of the three study regions. In Norway, no experiment in lakes receiving low levels of N deposition provided evidence of phytoplankton P limitation, but 12 of 19 experiments indicated a primary N limitation (Table 1 and table S2). In contrast, under elevated N deposition, no experiment produced any sign of N limitation, whereas 13 of 18 suggested phytoplankton P limitation. RRs representing the impact of N or P limitation on phytoplankton biomass also showed similar trends with N deposition levels (Table 1). The strength of this contrast is somewhat surprising, because N deposition levels in the low-deposition region of Norway that we studied ($\sim 4.5 \text{ kg N ha}^{-1} \text{ year}^{-1}$) are actually somewhat elevated relative to natural background levels found further north (16).

In Colorado, similar overall trends were observed; phytoplankton growth in high-N deposition lakes was P-limited, whereas in low-N deposition lakes, it was primarily N-limited (Table 1) (17). Although constrained to a shorter sampling time, spring and early summer experiments in Sweden (14) also showed signs of the same pattern seen in Norway and Colorado (Table 1). In all the sampled areas, response ratios were generally consistent with an ecological impact of distorted N:P supplies caused by atmospheric N inputs (Table 1). The relative balance of phytoplankton N versus P limitation (RR-N/RR-P) was inversely related to the lake TN:TP ratio (Fig. 2B). Below a TN:TP value of ~ 44.2 (by atoms), the large majority of experiments indicated N-limited phytoplankton growth (RR-N/RR-P > 1), and the lakes were entirely in low-deposition areas. Above a TN:TP value of ~ 110 , phytoplankton were consistently P-limited, and the lakes were almost entirely (with one exception) in high-deposition areas. Alterations in lake N:P stoichiometry brought about by atmospheric N deposition have therefore produced similar shifts in phytoplankton nutrient limitation across a wide variety of lakes on two continents.

Whereas overall effects of N deposition were generally similar for the three geographic regions

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considered, the consistency and magnitude of impact did vary somewhat both between and among regions. Differences in the impact of N deposition between regions may reflect variable N-loading gradients between high- and low-deposition areas; however, the similarity in the N-deposition gradients in the three study regions (Table 1) suggests that this is unlikely to explain differences in the apparent effect of N deposition on phytoplankton nutrient limitation. It seems more likely that between-region differences reflect contrasts in the relative impact of watershed vegetation on intercepting N. In Sweden,

lakes were located in forested catchments, whereas watersheds of lakes in Norway and Colorado were generally unforested. This may explain why the effects of N deposition on phytoplankton nutrient limitation in Sweden were somewhat modest compared with the effects seen in Colorado and especially Norway; differences in N/P limitation between high- and low-deposition areas in Sweden appear to be confined to the early growing season, when forest uptake of inorganic N is limited (14). Within-region variations may reflect various local factors that impinge on overall N and P supply and

loss rates, such as those associated with geological substrata, flow paths taken by inflow water, or the extent of wetland or lake denitrification (16).

Our findings show that, despite the potential of watershed vegetation uptake and sediment denitrification to buffer lakes against elevated N loading, increased inputs of anthropogenic N have accumulated in receiving waters. As a result, shifts in lake N:P stoichiometry have altered ecological nutrient limitation of phytoplankton growth. Phytoplankton in lakes that are less influenced by anthropogenic inputs experience relatively balanced

Table 1. Effects of atmospheric N deposition on average nutrient concentrations and ratios as well as on quantitative and qualitative phytoplankton responses to N or P enrichment in three regions. High- and low-deposition lakes are compared for each region and overall (via *t* test for quantitative parameters or chi-squared test for type of nutrient limitation). Quantitative measures are given as the

average RRs for enrichment by N or P or N and P (13). The type of limitation indicates the relative frequency of primary limitation or sequential colimitation by N or P as described in the supporting online material. n.s., not significant. *P* values in parentheses indicate the results of a *t* test comparing mean values for high- and low-deposition lakes for each region or for the entire study.

Country	Deposition	Number of lakes	NO ₃ (μM)	TN (μM)	TP (μM)	TN:TP (atomic)	RR-N	RR-P	RR-N/RR-P	Type of limitation	
										N	P
Norway	Low (~4.5 kg/ha)	19	0.11	9.81	0.34	32.8	1.64	1.03	1.63	12/19	0/19
	High (~8.5 kg/ha)	18	20.4	26.7	0.17	165	1.04	1.86	0.60	0/18	13/18
			<i>P</i> < 0.0001	<i>P</i> < 0.0001	<i>P</i> < 0.0001	<i>P</i> < 0.0001	<i>P</i> < 0.0001	<i>P</i> < 0.0001	<i>P</i> < 0.0001	<i>P</i> < 0.0001	<i>P</i> < 0.0001
United States	Low (~2 kg N/ha)	20	4.27	7.54	0.16	52.9	1.32	1.35	1.24	5/20	4/20
	High (~7 kg N/ha)	16	11.9	16.5	0.15	126	0.97	1.49	0.72	1/16	9/16
			<i>P</i> < 0.0001	<i>P</i> < 0.0001	n.s.	<i>P</i> < 0.0006	<i>P</i> < 0.02	(<i>P</i> < 0.06)	<i>P</i> < 0.03	(<i>P</i> ~ 0.12)	<i>P</i> < 0.03
Sweden	Low (~2 kg/ha)	7	0.12	8.02	0.25	34.5	1.32	0.96	1.39	3/7	0/7
	High (~6 kg/ha)	7	0.89	14.2	0.24	60.2	1.18	1.19	1.04	0/7	3/7
			<i>P</i> < 0.011	<i>P</i> < 0.01	n.s.	<i>P</i> < 0.001	(<i>P</i> ~ 0.14)	<i>P</i> < 0.001	(<i>P</i> < 0.09)	(<i>P</i> < 0.10)	(<i>P</i> < 0.10)
Total	Low	46	2.01	8.79	0.25	42.2	1.32	1.13	1.42	20/46	4/46
	High	41	13.6	20.1	0.17	131	1.18	1.54	0.72	1/41	25/41
			<i>P</i> < 0.0001	<i>P</i> < 0.0001	<i>P</i> < 0.001	<i>P</i> < 0.0001	<i>P</i> < 0.0001	<i>P</i> < 0.001	<i>P</i> < 0.0001	<i>P</i> < 0.0001	<i>P</i> < 0.0001

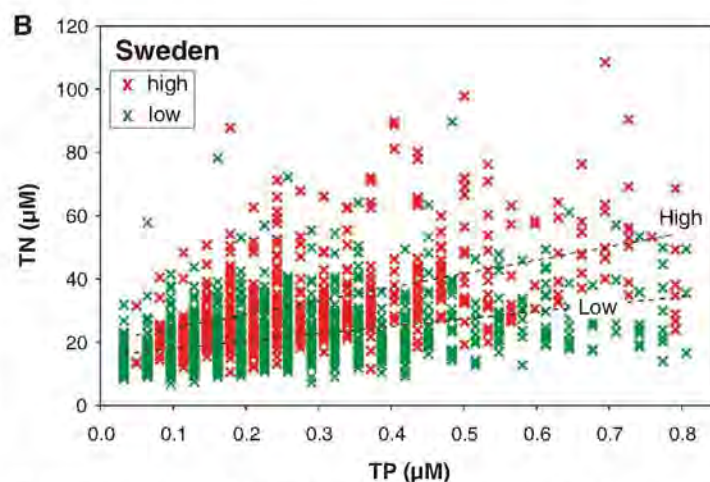
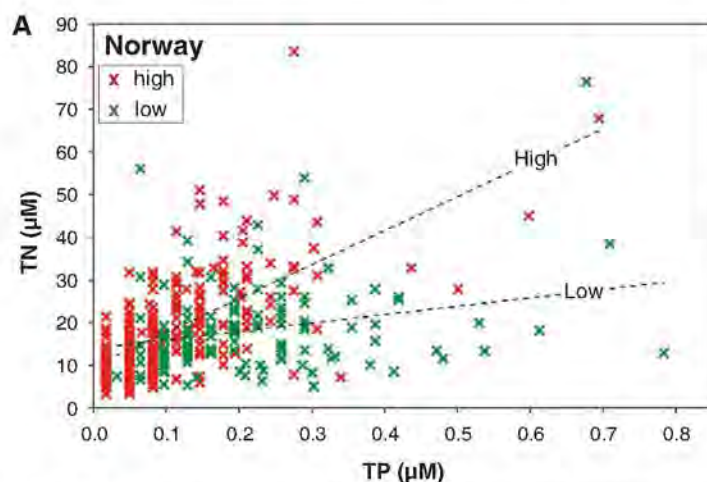


Fig. 1. The effect of atmospheric N deposition on lake N:P stoichiometry in Scandinavia. Compared to low-deposition lakes (green), lakes receiving high atmospheric N deposition (red) have higher TN concentrations for a given level of TP in both Norway (A) and Sweden (B). All relationships were highly significant

(*P* < 0.0001 and *R*² = 0.16 to 0.40). The slopes for TN versus TP relationships for the high- and low-deposition lakes were 76 and 32, respectively (Norway) and 43 and 23, respectively (Sweden). For clarity, values for TP for high-deposition lakes were offset by −0.015 μM so that they did not overlap with low-deposition data.

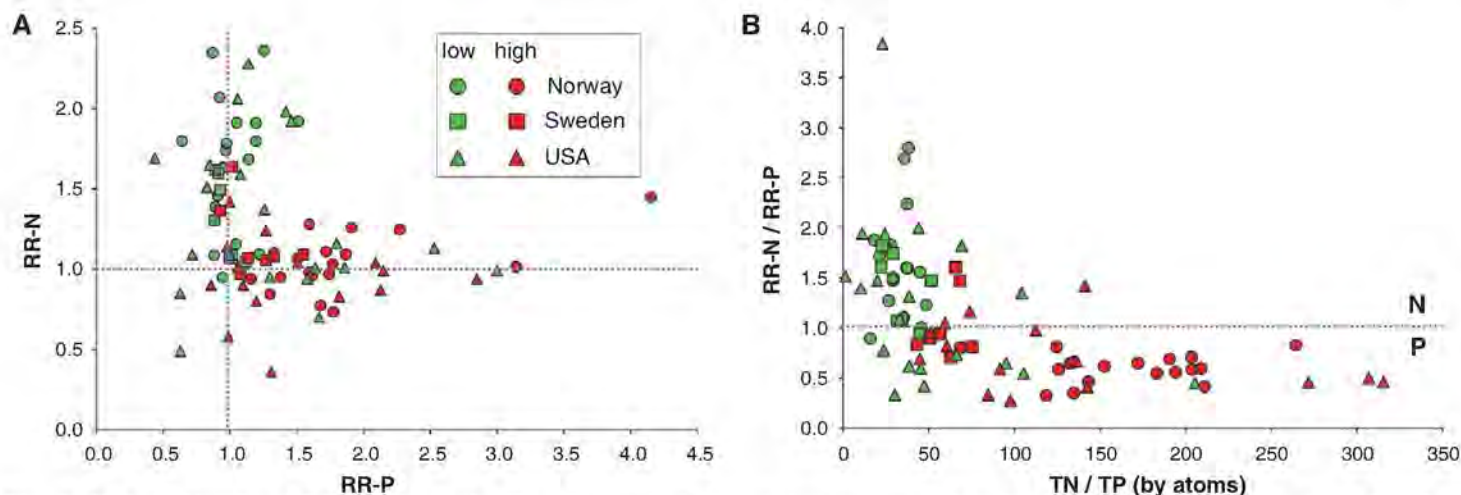


Fig. 2. Phytoplankton N and P limitation as a function of atmospheric N deposition in lakes of Norway (circles), Sweden (squares), and Colorado (triangles). Lake phytoplankton that respond strongly to N have a weak response to P and vice versa (A). Horizontal and vertical lines delineate response ratios of 1, indicating no response of phytoplankton biomass to enrichment of that nutrient. Results from low-deposition lakes (green) are

clustered on the y axis, indicating primary N limitation, whereas those from high-deposition lakes (red) are clustered on the x axis, indicating primary P limitation. The relative phytoplankton response to N compared with P (RR-N/RR-P) is strongly dependent on lake TN:TP ratio (B), which itself is dependent on N deposition. Values greater than 1 indicate that N limitation predominates in that lake, whereas values less than 1 indicate that P limitation predominates.

or N-deficient nutrient supplies, but enhanced N inputs from the atmosphere during the past several decades of human industrialization and population expansion appear to have produced regional phytoplankton P limitation. Producer diversity is likely to be low when resource supply ratios are skewed in favor of one particular nutrient relative to others (11, 18). Thus, increased N loading from the atmosphere may reduce lake phytoplankton biodiversity, similar to anticipated effects of N deposition on plant diversity in terrestrial ecosystems (19, 20), by possibly favoring those relatively few species that are best able to compete for the limiting P. Enhanced phytoplankton P limitation may also impair food-web performance, because both laboratory and field studies (21) have shown that P-limited algae are poor-quality food for zooplankton because of unsuitable biochemical composition (22) or low P content (23). Indeed, this is possible in the Colorado and Norway lakes that we studied, in which particulate biomass (seston) C:P and N:P ratios were significantly higher ($P < 0.006$) in high-deposition lakes (240 versus 197 C:P by atoms, and 36.2 versus 26.7 N:P by atoms). Thus, sustained N deposition that generates stoichiometric imbalance between P-limited, low-P phytoplankton and their P-rich zooplankton consumers (12) may result in reduced production of higher trophic levels, such as fish. Projected increases in global atmospheric N transport during the coming decades (24) are likely to substantially influence the ecology of lake food webs, even in lakes far from direct human disturbance.

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Supporting Online Material

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Materials and Methods
Tables S1 and S2
References

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Abiotic Gas Formation Drives Nitrogen Loss from a Desert Ecosystem

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In arid environments such as deserts, nitrogen is often the most limiting nutrient for biological activity. The majority of the ecosystem nitrogen flux is typically thought to be driven by production and loss of reactive nitrogen species by microorganisms in the soil. We found that high soil-surface temperatures (greater than 50°C), driven by solar radiation, are the primary cause of nitrogen loss in Mojave Desert soils. This abiotic pathway not only enables the balancing of arid ecosystem nitrogen budgets, but also changes our view of global nitrogen cycling and the predicted impact of climate change and increased temperatures on nitrogen bioavailability.

After the presence of water, nitrogen availability is the primary constraint to biological activity in many arid ecosystems (1). Despite the existence of large pools of

soil N, arid regions such as deserts, dry shrublands, and savannas often lack bioavailable forms of N (2, 3). Inputs and losses of biologically available N therefore directly affect ecosystem

productivity. Gaseous emissions from soil are thought to be the primary form of N export in arid lands and are estimated to be as much as 1 to 3 kg ha⁻¹ year⁻¹ in arid regions of North America and Africa (4, 5). To date, annual estimates of N loss from deserts have been calculated based on measurements of inputs and storage and have included little direct characterization of N loss dynamics. It has been assumed that differences between N inputs and storage can be attributed to microbial activities that produce trace N gases at the soil surface, with emissions being driven by nitrification and denitrification during the growing season (6). Recent studies have shown, however, that N loss patterns are inconsistent with biological mechanisms of N gas production. For example, of the ~1 kg ha⁻¹ year⁻¹ estimated N loss in the northern Mojave Desert in the form of reactive N gases (i.e., nitrogen oxides and NH₃), ~75% occurs in the summer under conditions hostile to biological activity (7). Abiotic formation of N gases may therefore be the dominant form of N export in the Mojave Desert and is likely to drive N loss in most arid ecosystems. Identification and quantification of this flux will potentially account for the N imbalance in arid regions (8).

We used field and laboratory approaches to monitor production of reactive N from Mojave Desert soils and to describe the underlying mechanisms of this production. In this context, we defined reactive nitrogenous gases as nitrogen oxides (NO_x, all forms of oxidized gaseous N) and NH₃. We excluded less reactive chemical species (for example, N₂ and N₂O), as they have previously been measured or estimated and shown to be a minor component of N loss in this and other arid regions (9, 10). Summer in the Mojave Desert yields extreme diurnal patterns in light and soil temperature that allowed us to test abiotic formation of reactive N (11). We monitored total reactive N fluxes (segregated as NO_x, other forms of NO_x, and NH₃), starting before dawn and continuing until after dark in both naturally dry soils and 24 hours after an artificial 25-mm rain event. Measurements were replicated across the range of soil heterogeneity typical of deserts (12) and included both plant interspaces and soils influenced by the dominant shrub *Larrea tridentata*. To further explore the effect of solar radiation on abiotic formation of reactive N, we used radiation shields in the field to induce short-term flux responses to the complete removal of light and reductions in soil temperature associated with solar radiation. These field manipulations of solar radiation, however, did not allow for the separation of the direct (light) and indirect (temperature) effects of solar radiation on the chemical formation of reactive N gases. To identify the individual effect of temperature on re-

active N gas emissions, we manipulated surface temperatures of intact soil profiles from 15° to 75°C under constant light and measured the production of nitrogen oxides and ammonia as well as soil respiration. Measurements were made on soils collected from interspaces covered in biological soil crust and from beneath *L. tridentata* and were either dry or watered to field capacity and allowed to dry for 60 hours.

In the field, we attributed production of reactive N from dry soils to abiotic processes and, additionally, fluxes from wet soils during mid-

day peaks in radiation and soil temperature [mid-day ground temperatures during measurements were >65°C and can exceed 90°C in the Mojave Desert (13)] were also attributed to nonbiological sources. Diurnal measurements showed strong daily patterns of reactive N gas efflux, with emissions peaking at midday when soil radiation approaches 1000 W m⁻² and surface soil temperatures exceed 60°C (Fig. 1) (14). Maximum fluxes from dry soils were 5.5 ng N m⁻² s⁻¹. This value is similar to flux estimates (consisting only of NO) attributed to biological nitrification

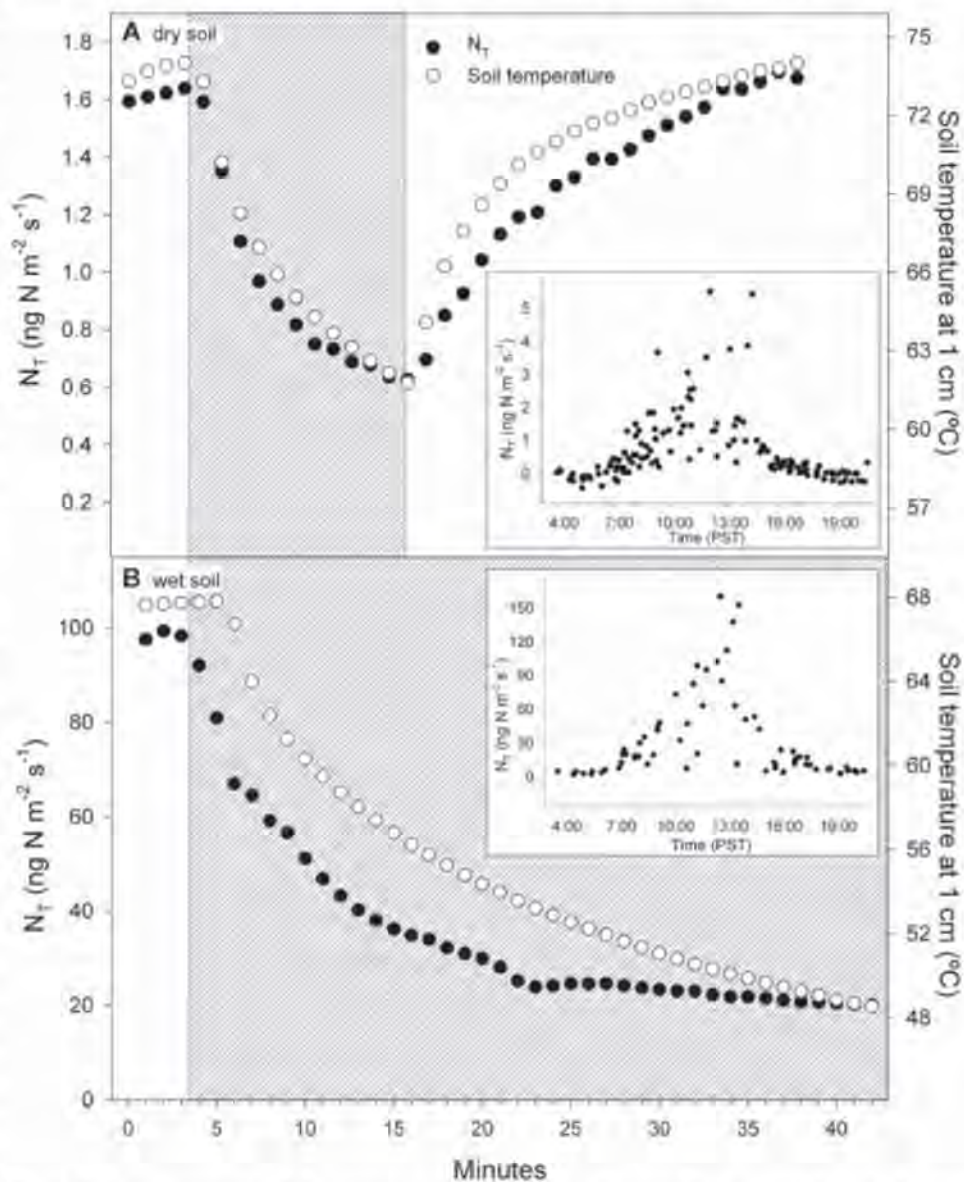


Fig. 1. Soil measurements of the response of total reactive nitrogen (N_T) gas emissions to changes in solar radiation from (A) dry soil and (B) wet soil, 24 hours after an artificial 25-mm rain event. The main panels show the responses of N gas fluxes and soil temperature in plant interspaces during the removal of all incoming radiation; shaded areas indicate periods when all incoming solar radiation was blocked with a radiation shield. Shading responses were measured at midday, between 12:00 and 2:00 p.m. (Insets) The diurnal pattern of reactive N gas emissions during the summer across a heterogeneous landscape under dry (A) and wet (B) soil conditions. Each data point is an individual measurement, and the diurnal pattern includes data points collected on multiple days during two successive summers (2007 and 2008). The composition of the flux differed between the two treatment conditions. Fluxes from dry soils were 19% NO_x, 55% other forms of NO_x, and 26% NH₃. In contrast, emissions from wetted soils were 62% NO_x, 25% other forms of NO_x, and 13% NH₃. PST, Pacific Standard Time.

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reported in tropical woodlands that are thought to have some of the highest rates of biological reactive N gas production (15). A diurnal pattern was also clearly present in recently wetted soils (Fig. 1). However, the response was several orders of magnitude larger than that in dry soils with fluxes reaching $160 \text{ ng N m}^{-2} \text{ s}^{-1}$, a rate comparable to some of the largest pulses of biological NO reported for natural ecosystems (16). The continued presence of a diurnal flux pattern in wet soils contrasts with other studies of post-rain measurements of N gas efflux (17, 18), in which fluxes rapidly decline after an initial pulse, and emphasizes the role of abiotic processes in describing N gas loss patterns from Mojave Desert soils. Light removal experiments conducted during the midday peak in reactive N gas efflux on interspace soils yielded conclusive evidence for the importance of solar radiation in driving N gas loss from desert soils. There was an immediate decrease in both reactive N gas emissions and soil temperature at 1 cm in response to changing light conditions, and the removal of light resulted in significant reductions in total reactive N gas efflux from both wet and dry soils ($P < 0.05$) (Fig. 1). The same response was recorded for soils beneath *L. tridentata* (table S1). The temporal offset between the response in N gas emission and temperature observed in wet soils is probably due to the slight delay in tem-

perature propagation to the depth of the temperature measurement. The immediacy of this response makes it unlikely that flux reductions are driven by changes in soil N availability associated with autotrophic N fixation; instead, the response appears to be closely linked to light-dependent changes in surface soil temperature.

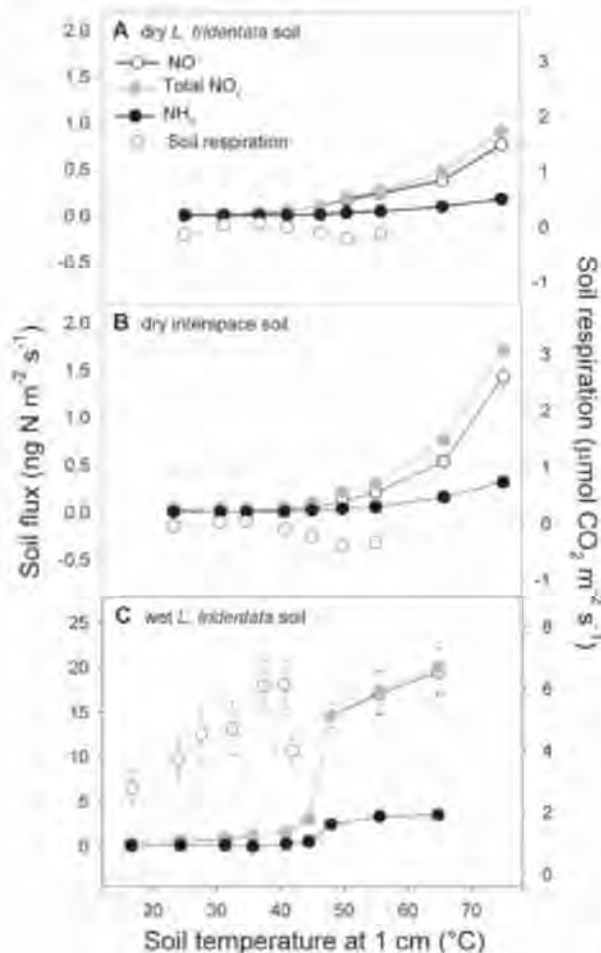
The correlation between surface soil temperature and reactive N gas efflux was further supported by the results of laboratory manipulations in which temperature was varied under constant, low-level light conditions ($350 \mu\text{E}$) (Fig. 2). In dry soils, reactive N gas fluxes increased ($P < 0.0001$) with surface soil temperature for both cover types (Fig. 2), with losses increasing from 0.05 to $2 \text{ ng N m}^{-2} \text{ s}^{-1}$ over a temperature range of 20° to 70°C . Soil temperatures of 40° to 50°C were an important threshold for this response: Lower temperatures had little effect on fluxes, but higher temperatures had a strong positive effect on fluxes. No detectable soil respiration occurred in dry soils, indicating that the N gas production is driven exclusively by abiotic mechanisms. Wet soils showed a similar response to increasing temperature ($P < 0.0001$); however, fluxes were one order of magnitude higher (Fig. 2C). In wet soils, respiration showed a positive correlation with temperature up to 40°C , abruptly declined as temperatures exceeded 40°C , and was then unmeasurable by our system at tem-

peratures $>50^\circ\text{C}$. Differential responses of soil respiration and reactive N gas production to changing soil temperature support the hypothesis that N gas emissions are driven by nonbiological processes. Respiration was not assessed at the highest temperatures ($>50^\circ\text{C}$) because the CO_2 monitoring equipment would not operate at these temperatures, but multiple studies have shown that microbial processes that produce reactive N are inhibited at temperatures exceeding 40°C (19–21). Thus, biological activity is very unlikely to substantially contribute to the flux of reactive N during these periods of high reactive N gas loss.

Our results show that abiotic reactions are a key component of N loss from desert soils, both under dry conditions and during post-wetting periods. Not only is there a continuous chemical loss of N from dry soils, but after soil wetting there is a substantial increase in production of reactive N gas that cannot be attributed entirely to biological activity. This increased loss rate is probably a combined effect of higher concentrations of biologically produced substrate and a faster rate of reactions when substrates more readily enter solution. Such high rates of abiotic N gas loss from both wet and dry soils suggests that traditional measurement approaches that avoid dry soil and summertime conditions underestimate total N gas loss from desert soils and may misrepresent patterns and sources of these gases. Abiotic decomposition of HNO_2 to NO (chemodenitrification) can drive N gas loss in soils. However, it occurs exclusively in wet, acidic soil microsites (22) and is therefore unlikely to explain the abiotic N gas production observed in the highly alkaline soils used in this study. Thus, the observation reported here of abiotic nitrogen gas production from desert soils identifies a gap in our understanding of this process. The mechanism is probably high-temperature oxidation of organic and/or inorganic nitrogen. However, these processes have only been shown to occur at higher temperatures (23, 24) and now must be considered to potentially occur at lower temperatures.

In addition to dominating arid-land N export, soil emissions of N gases yield reactive nitrogenous chemical species (e.g., NO, NO_2 , and NH_3) with relatively short atmospheric lifetimes that drive several aspects of regional air quality, including ozone chemistry and aerosol formation (25). In contrast to less reactive N gases, such as N_2O and N_2 , that are stable in the troposphere, the reactivity of these chemical species often means that local sources, including soil fluxes, drive atmospheric chemistry on a regional scale (26). This is especially true in remote regions. For example, in Antarctica and other isolated snow-covered areas, photochemical reactions in snow yield N gases that affect the oxidizing capacity of the troposphere (27). Such fluxes of reactive N peak around $0.3 \text{ ng N m}^{-2} \text{ s}^{-1}$ (28), less than 20% of midday emissions into the atmosphere above the Mojave Desert. Given the

Fig. 2. Laboratory measurements of the response of reactive N gas emissions and soil respiration to changes in soil temperature in (A) dry soil from beneath *L. tridentata*, (B) dry soil from plant interspaces, and (C) wetted soil from beneath *L. tridentata*.



magnitude of desert fluxes relative to those found to be important in polar regions, it follows that the abiotic reactions identified in this study can play an important role in atmospheric processes above remote arid sites.

The high proportion of reactive N gas efflux associated with abiotic sources suggests that, similar to carbon released during photodegradation of litter (29), N in arid environments can bypass biotic pools by returning directly to the atmosphere via abiotic pathways. This decoupling of nutrient dynamics from biological processes in water-limited ecosystems means we must consider how arid environments may respond to global change. Carbon dynamics in water-limited ecosystems may be most sensitive to global change factors that alter the composition and intensity of radiation reaching Earth's surface (18). Our study not only identifies N cycling as being equally sensitive to solar radiation as carbon, but also as highly temperature-dependent. Ultimately, rates of N export from arid ecosystems will be determined by summer surface soil temperatures and the frequency of summer precipitation events, both of which are predicted to change under most scenarios of future global climate (30). The conditions that promote abiotic N gas loss from desert soils also inhibit N fixation (31), the dominant N input to arid regions. Future environmental shifts will therefore probably disrupt natural N dynamics by unbalancing rates of N inputs and losses, gradually reinforcing N limitations in arid environments.

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Supporting Online Material

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Materials and Methods

Table S1

References

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A Probable Pollination Mode Before Angiosperms: Eurasian, Long-Proboscid Scorpionflies

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The head and mouthpart structures of 11 species of Eurasian scorpionflies represent three extinct and closely related families during a 62-million-year interval from the late Middle Jurassic to the late Early Cretaceous. These taxa had elongate, siphonate (tubular) proboscides and fed on ovular secretions of extinct gymnosperms. Five potential ovulate host-plant taxa co-occur with these insects: a seed fern, conifer, ginkgoopsid, pentoxylean, and gnetalean. The presence of scorpionfly taxa suggests that siphonate proboscides fed on gymnosperm pollination drops and likely engaged in pollination mutualisms with gymnosperms during the mid-Mesozoic, long before the similar and independent coevolution of nectar-feeding flies, moths, and beetles on angiosperms. All three scorpionfly families became extinct during the later Early Cretaceous, coincident with global gymnosperm-to-angiosperm turnover.

Animal pollination, most frequently accomplished by insects (1), benefits seed plants by ensuring efficient fertilization without relying on costlier, abiotic modes such as wind and water transport (2). In return for pollination, plants provide a variety of nutritional and other rewards to pollinating animals, particularly pollen and nectar (1). Little is known about

pollination mutualisms during the mid-Mesozoic (3, 4)—before the origin of flowering plants in the Early Cretaceous (5)—although evidence from the fossil record of insect mouthpart structure (6), gut contents (7), insect consumption of plant reproductive organs (8), and plant reproductive features (4, 9) indicates earlier consumption of pollen, nectar-like fluids, and other plant

tissues (8). Here, we show that a major clade of extinct, siphon-bearing, fluid-feeding insects, early members of the order Mecoptera (scorpionflies), were likely feeding on gymnosperm ovulate secretions during the late Middle Jurassic to mid-Early Cretaceous. Modern scorpionflies are a relict clade of ~600 species, of which the relict Nannochoristidae have fluid-feeding mouthparts that are inferred to imbibe nectar and other plant exudates (10), perhaps representing an underappreciated legacy from distant Mesozoic ancestors. In contrast to some of their antecedents, modern Mecoptera overwhelmingly are minor predators or consumers of dead organisms (11) and rarely are implicated as floral visitors (12).

Our material consists of 21 insect specimens, six newly described (13), representing 11 species, five genera, and three families of Eurasian Mecoptera (tables S1 and S2). This mid-Mesozoic group encompasses the families Mesopsychidae (Fig. 1), Aneuretopsycheidae (Fig. 2, A to E and G to I, and fig. S2), and Pseudopolycentropodidae (Fig. 2, F and J to O), for which the encompassing name Aneuretopsychina is available (14). These three lineages likely originated during the Late Permian, as judged by the co-occurrence of several closely related lineages during this epoch (15, 16). Taxa of these long-proboscid lineages are uncommon but persistent in Eurasian deposits representing an interval of 62 million years

(My), from the late Middle Jurassic (Bathonian-Callovian boundary interval) to the late Early Cretaceous (late Albian Stage). We examined adpression specimens from four fine-grained deposits of late Middle Jurassic and Early Cretaceous ages, as well as one specimen from late Early Cretaceous amber (13).

We conducted a cladistic analysis using 53 morphological characters, 19 extinct taxa, and 12 extant taxa (tables S3 to S5). In the best-supported cladogram (Fig. 3 and fig. S1), the three siphonate lineages—Mesopsychidae, Aneuretopsycheidae, and Pseudopolycentropodidae—strongly support monophyly of the Aneuretopsycheina (16), with unique head and mouthpart apomorphies, but retain generalized symplesiomorphies for most other characters, such as wing venation, leg characters, and genitalic structure. The inclusion of basal Permochoristidae (as Pseudonannochoristinae) and a terminal Permotipulidae in the Aneuretopsycheina, currently based on wing features, suggests siphonate mouthparts in these taxa. We consider the Aneuretopsycheina the sister taxon of the remaining, dominantly modern Mecoptera, rather than a stem group or paraphyletic lineage as previously proposed (16).

Excluding appendages, the 11 species range in body length from 3 mm in *Parapolycentropus burmiticus* to 28 mm in *Lichnomesopsyche gloriæ* Ren, Labandeira & Shih gen. et sp. nov. (13, 17) (Fig. 1A). Wing venation indicates that these insects were modest flyers and could not hover, similar to modern scorpionflies but unlike some coeval nematocerous flies (8, 18). The heads are rather small and triangular or subcircular in frontal aspect, with large but nonconverging spheroidal, compound eyes; filiform, pectinate, or moniliform antennae; and a distinctive clypeal sclerite indicating an underlying cibarial sucking pump. The external mouthparts in all but one species bear structures typical of extant, long-proboscid, pollinating insects (19) and prominent, long, siphonate mouthparts with or without accompanying maxillary palps. The prolonged tubular siphons are labial in origin and anatomically constitute the two sutured halves of the labial palps that are anchored at the premental base (19, 20). Many of the labial siphons bear diverse external cuticular ornamentation and vestiture, various terminal processes associated with

feeding, and a subterminal circular to ellipsoidal mouth. Conspicuously absent are the downward extension of the head capsule and a terminally located, complete series of mandibulate mouthparts typical of modern Mecoptera (11, 20).

Representing the Mesopsychidae is *L. gloriæ* (13) (Fig. 1, A to N, and table S2), our most fully documented species. Its head is triangular, posteriorly clothed with stiff setae between moderately bulging eyes; the head lacks ocelli and has a prominent, trapezoidal, clypeal sclerite situated immediately below the insertion of compact, filiform, tapering antennae (Fig. 1, B to D). The base of the siphon internally is characterized by distinctive transverse costae lining the outer food-tube surface, suggesting a second dilatory pump (Fig. 1I). Three-segmented maxillary palps are present on either side of a narrow labrum and proboscis base, all of which support local fields of moderately coarse setae and bear a falcately shaped third segment (Fig. 1, D and E). The forwardly directed, smooth surfaced siphon is 9.3 mm long (all dimensions herein reported are arithmetic averages; $\sigma = 0.033$, $N = 6$), 0.28 mm in unreconstructed diameter ($\sigma = 0.33$, $N = 7$), and houses a food tube 0.128 mm in unreconstructed diameter ($\sigma = 7.33 \times 10^{-3}$, $N = 7$). The siphon is covered by a vestiture of setae arranged in a distinctive chaetotaxic pattern along its lateral margins (Fig. 1, E, F, L, and N), deployed as nearly parallel longitudinal rows of similarly oriented setae. These setae occur from the proboscis base to the terminus, but they are absent from the immediate region of the subterminal circular mouth and at the ventrolaterally positioned, ovoidal labial pads (Fig. 1, F, J, L, and N), here termed pseudolabellae (type 1), which consist of fleshy tissue for likely capillary uptake of fluids. We interpret the siphon as exhibiting moderate flexibility and supported by ~10 flexible internal strands surrounding the food canal (Fig. 1, J and K). We infer that musculature, originating from the head capsule and attached along the proboscis, was responsible for siphonal flexion and directional control.

Congeneric with *L. gloriæ* is *L. daohugouensis* Ren, Labandeira & Shih sp. nov. (13) (fig. S3 and table S2), a smaller, rarer species of half the body length (14 mm) and bearing a shorter siphon covered with a vestiture of dense, fine setae. The two studied specimens have a siphon that is 8.8 mm long, with an unreconstructed mean diameter of 0.325 mm and a food tube diameter of 0.105 mm. A larger, incompletely preserved, yet different mesopsychid species with a more ellipsoidal head is *Vitimopsyche kozlovi* Ren, Labandeira & Shih gen. et sp. nov. (13) (Fig. 1, O to R, and table S2). This single specimen bears distinctive pectinate antennae (Fig. 1R), broad, laterally placed eyes, and a rectangularly shaped clypeus (Fig. 1, P and Q). The distinctive, forwardly deployed siphon, 9.0 mm long, has a restored width of 0.58 mm, contains a food canal 0.14 mm in diameter, and lacks pseudolabellae (table S2). The siphon is preserved as two sep-

arated labial halves, each part of which has a dark, longitudinal ridge that is matched by an analogous but offset ridge on the counterpart, indicating an interlocking tongue-and-groove attachment mechanism. These longitudinal structures are unlikely to be stylets (which would be considerably more robust, sclerotized, and continuous), would exhibit a separate and partly nonparallel position within the opened labial siphon, and would display modifications for tissue penetration (such as retorse or serrated lateral edges or an acuminate tip). The absence of external cuticular ornamentation or pilosity on the siphon may be attributable to iron mineral replacement, disallowing preservation of fine detail; alternatively, the proboscis of *V. kozlovi* may have been unornamented in life.

The Aneuretopsycheidae bore differently shaped heads, downwardly deployed probosces, and distinctively ornamented siphonal surfaces that lacked setae. *Jeholopsyche liaoningensis* Ren, Shih & Labandeira gen. et sp. nov. (13) (Fig. 2, A to E, fig. S2, and table S2) is a superbly preserved specimen of medium body size, 24 mm long, with a head and proboscis whose dorsal aspect is preserved on the ventral side of the insect. The head is transversely oriented and stoutly ellipsoidal, has compact filiform antennae, prominent ocular sclerite encompassing large circular eyes positioned from the ventral to dorsal portion of the head capsule for a wide visual field, and lacks palps (Fig. 2, B and C). The distinctively shaped clypeus housed an inner cibarial pump, as determined by positional homology in a wide variety of modern scorpionfly taxa. The distinctively annulated siphon lacks setae, is 6.8 mm long and 0.34 mm wide, and has walls that expand at its base, below the clypeus, indicating a possible second dilatory suction pump, but positioned differently than in *L. gloriæ*. Along the length of each transverse annulus is a series of perpendicularly arranged, short ridges. The siphonal terminus (Fig. 2, D and E, and fig. S2) bears a subterminal ellipsoidal mouth containing an opaque substance and is partly surrounded terminally by a V-shaped pad and by lateral fleshy pseudolabellae (type 2) that extend beyond the siphonal margin. These and other pseudolabellae probably are homologous with the functionally similar, distal palpal patch of modern Mecoptera and perhaps the labellum of Diptera (true flies). A conspicuous food tube, 0.10 mm in diameter, contains occasional blobs of opaque material identical to a nonparticulate substance lodged in the mouth (fig. S2). A second aneuretopsycheid specimen, *Jeholopsyche* sp. (Fig. 2, G to I), similarly has a downturned proboscis as in other Aneuretopsycheidae and Pseudopolycentropodidae, rather than a siphon directed anteriorly, as in the Mesopsychidae. *Jeholopsyche* sp. has medium-sized eyes, but its head shape, antennae, and absence of palps are similar to those of *J. liaoningensis*. The siphon is 5.8 mm long, 0.28 mm wide, and houses a food tube 0.085 mm in diameter (table S2), but

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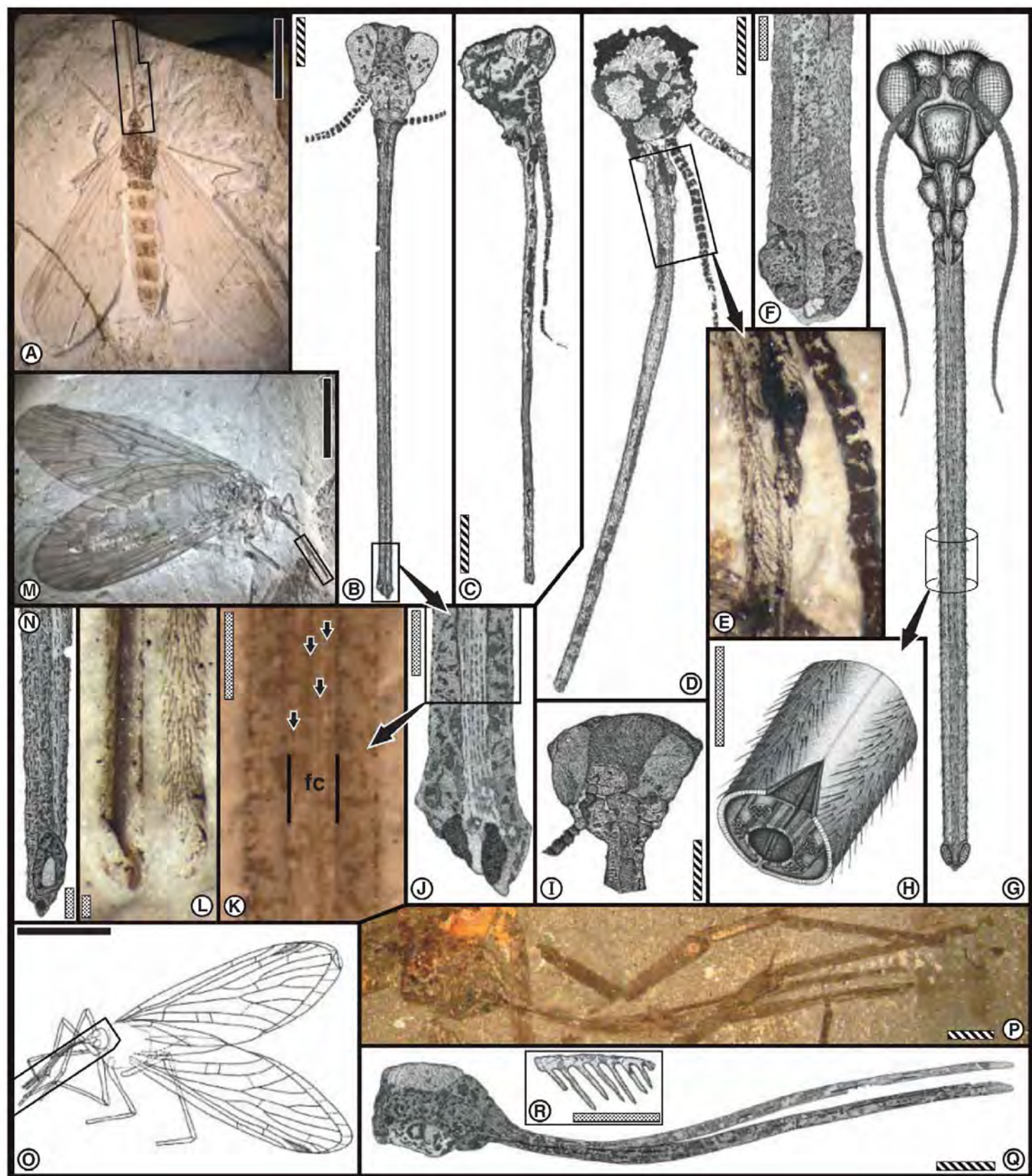
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in contrast to its congener, it lacks annuli and bears transverse, interrupted cuticular ridges that have regular spacing throughout the organ, and terminates in a unique, expanded pseudolabellum (type 3). The siphonal ridges are reminiscent of similar structures in some butterflies,

such as *Pieris* (20) or *Dryas* (21), suggesting a similar function for probing into confined structures of host plants.

The much smaller Pseudopolycentropodidae is represented by two specimens of *Pseudopolycentropus janeannae* Ren, Shih & Labandeira

sp. nov. (13) (Fig. 2, J to L, and table S2). This species has a triangularly shaped head, moderately-sized, circular compound eyes positioned laterally, a prominent labrum, compact filiform antennae, and a siphon in an anatomically downturned position lacking external cuticular ornamenta-



tion but presenting very fine setae, pushing the limit of preservational resolution. The siphon is ~1.6 mm in length and ~0.13 mm in diameter and lacks terminal structures related to feeding. The food canal, ~0.038 mm in diameter, is narrow for the proboscis width. A related larger specimen of *P. janeanae*, for which only one head and mouthpart ensemble have been examined, possesses an observable proboscis at least 1.9 mm long, displaying 160° of flexion without brittle fracture (Fig. 2F). The second pseudopolycentropodid species, amber *Parapolycentropus burmiticus* (Fig. 2, M to O), has a miniscule body length of 3.0 mm, a triangular head with prominent occipital setae, modest, laterally placed eyes, setate moniliform antennae, and a distinctive, robust labrum (17). The downwardly deflected proboscis is stylete rather than siphonate, annulated, has prominent setae arising from interannular sulci, and bears at least one protruding, robust stylet above two, elongate labial lobes that form pseudolabellae (type 4). The siphon is small, 1.33 mm long, 0.12 mm in width, and houses a barely detectable food canal 0.014 mm in width. *P. burmiticus* is the only species examined to bear stylete rather than siphonate mouthparts, consistent with at least some blood feeding (17). The thickened, buttressing labrum of *P. burmiticus* (Fig. 2, M and N) is interpreted as a major, force-absorbing brace analogous to the similarly sized, highly muscled, and anatomically homologous labrum in extant, blood-feeding mosquitoes (22). Extensive labral development in *P. burmiticus* is absent in the other examined fossil taxa; conversely, head and mouthpart features of the aneuretopsychine mouthparts are consistent with passive, nonpenetrative uptake of surface-accessible plant fluids.

Modern, long-proboscate, fluid-feeding dipteran lineages include nonstylete, nectar-feeding individuals that often are pollinators, as well as stylete, blood-feeding individuals. Blood-feeding taxa occur either as different species of the same higher-level taxon or as male (nectar-feeding)

versus female (blood-feeding or both) sexes of the same species (23). This dual feeding strategy in several modern fly lineages may be analogous to some Early Cretaceous pseudopolycentropodids. By contrast, the presence of siphonate proboscides in the 10 other mecopteran taxa examined is likely homologous with the newly documented head and mouthpart structures of the most ancient, extant lineage of scorpionflies: the poorly known, relict, Gondwanan Nannochoristidae (10), which has a fossil record extending to the Early Jurassic (16), including thousands of specimens from the Middle Jurassic of China. Modern nannochoristids provide an anatomical context and valuable morphological link for assessing mouthpart differences between the Mesozoic Aneuretopsychina (Fig. 3) and modern Mecoptera.

Extant Nannochoristidae have been assigned variously to a basal lineage within modern Mecoptera, a separate order of insects, a sister group to Diptera, or to a clade including the Siphonaptera (fleas), among other options (10, 24). The feeding apparatus of extant Nannochoristidae is unique among extant scorpionflies. Fluid feeding apparently consists of initial uptake by capillary forces at the labial palp tip (the contact organ) followed by suction through modification of elongate, conjoined, mostly fleshy labial palps and labrum into a tubular structure. Each two-segmented palpus forms a half-tube that seals, together with the labrum, a short, projecting siphon powered by an enlarged pharyngeal pump centered under a frontal, quadrangular clypeus. In addition to tube-like elongation of the labium, there are other fluid-feeding, siphonate modifications shared between modern nannochoristid and extinct aneuretopsychine scorpionflies. These structures include a pilose and desclerotized proboscis outer surface, modification of the labial tip into a region of scales perhaps homologous with the aneuretopsychine pseudolabellum, absence of glossal and paraglossal medial labial lobes, reduction or loss of mandibles, expansion

of the pharyngeal chamber into a significant suction pump, probable strengthening of intrinsic palpal musculature, and external muscles originating from the internal head capsule. Unlike the more simplified aneuretopsychine proboscis, the nannochoristid condition also includes maxillary elements, principally an egesting salivary tube within an ingesting food tube. When compared to the nannochoristid condition (10), there is no evidence that the Aneuretopsychina ever included maxillary salivary structures within their proboscis, indicating considerable mouthpart reduction and simplification in early mecopteran evolution (24).

We scanned all examined insects under epifluorescence microscopy, particularly heads and mouthparts for surface pollen and food canals for ingested, lodged grains (13). Previous studies indicate that pollen occurs as insect gut contents (7) or rarely as clumps on the head and proboscis base, such as a brachyceran fly from another Eurasian site of mid-Early Cretaceous age (25). Our results were negative for both surface and food-canal pollen in the compression and amber material. The apparent absence of pollen may be due to taphonomic factors (e.g., relatively low carbon content in some specimens, relatively high oxidation level in the matrix of many of the specimens, loss of pollen during diagenesis); alternatively, the absence of pollen may be genuine. The head and mouthparts of the amber specimen were well preserved and readily imaged, so the lack of pollen likely reflects true absence rather than taphonomic artifact.

We also examined the chemical contents of the proboscis food canal from the best-preserved specimen, *J. liaoningensis* (Fig. 2, A to E), by energy-dispersive spectroscopy (EDS). We produced elemental results from EDS for four regions: the matrix surrounding the fossil, the femur of the right metathoracic leg, a targeted plug of food canal material approximately midway between the proboscis base and tip, and

Fig. 1. Head and siphonate mouthpart features associated with fluid-feeding Mesopsychidae scorpionflies from the mid-Mesozoic of northeastern China. (A to N) Six specimens of the new mesopsychid genus and species *Lichnomesopsyche glorie* Ren, Labandeira & Shih gen. et sp. nov. (13), from the late Middle Jurassic (transitional Bathonian-Callovian) Jiulongshan Formation of Inner Mongolia, China. (A) Specimen CNU-M-NN2005020-1. (B) Enlargement of template in (A) as an overlay drawing of head and mouthparts. (C) Overlay drawing of specimen CNU-M-NN2005024. (D) Overlay drawing of specimen CNU-M-NN2005021-1. These images show long, tapering, filiform antennae; head and proboscis setal patterns; large, separate compound eyes; medially adpressed, three-segmented maxillary palps; and proboscis base, indicating a cibarial pump. (E) Enlargement of basal proboscis region from rectangle in (D), exhibiting, from left to right, proboscis base with setal pattern, three-segmented left maxillary palp, and right antenna. (F) Overlay drawing of a proboscis terminus, showing the central food canal, subterminal mouth, and prominent, ventrolaterally placed, type 1 pseudolabellum [CNU-M-NN2005025, counterpart of (L) below]. (G) Reconstruction of head and mouthparts based on details of all specimens. (H) Midproboscis region indicated in (G) is sectioned to illustrate the external pilosity, medial food canal reinforced by longitudinal rodlike structures, and inferred muscles for control of proboscis terminus. (I) Head and detail of basal proboscis region (CNU-M-

NN2005029), exhibiting fine, transverse costae lining the food tube, probably representing a sucking pump. (J) Overlay drawing of a ventral proboscis (CNU-M-NN2005-025) from a scanning electron microscope (SEM) image of the proboscis tip in (B). (K) Enlargement of boxed area in (J), a composite of images from five tile scans using extended depth of field (EDF), illustrating the food canal (fc), reinforcing bundles (arrows), and proboscis margin, reconstructed in (H). (L) Photograph of ventral aspect of a proboscis terminus showing subterminal ventrolateral pseudolabellae, distinctive pilosity clothing the external surface, and probable food contents as a vertical, blobby line at left side of food canal [CNU-MM-2005-025, counterpart of (F) above]. (N) Lateral view of a ventrolateral terminal pad and mouth (CNU-M-NN2005-027-2), representing specimen tip in (M) outlined by a rectangle. (O to R) Specimen of a mesopsychid species, *Vitimopsyche kozlovi* Ren, Labandeira & Shih sp. nov. (13) (CNU-M-HP2005001), from the Early Cretaceous (Barremian) Yixian Formation of Hebei, northeastern China. (O) Camera lucida drawing. (P) Head and siphonate mouthpart region from rectangle in (O), enlarged as a SEM image integrating five EDF tile scans. (Q) Drawing of image in (P), showing formerly conjoined, subsurface, labial premental-palpal halves with possible tongue-and-groove interlocking. (R) Drawing of an associated pectinate antennal fragment occurring adjacent to the head in (O). Scale bars: solid, 10 mm; striped, 1 mm; dotted, 0.1 mm.

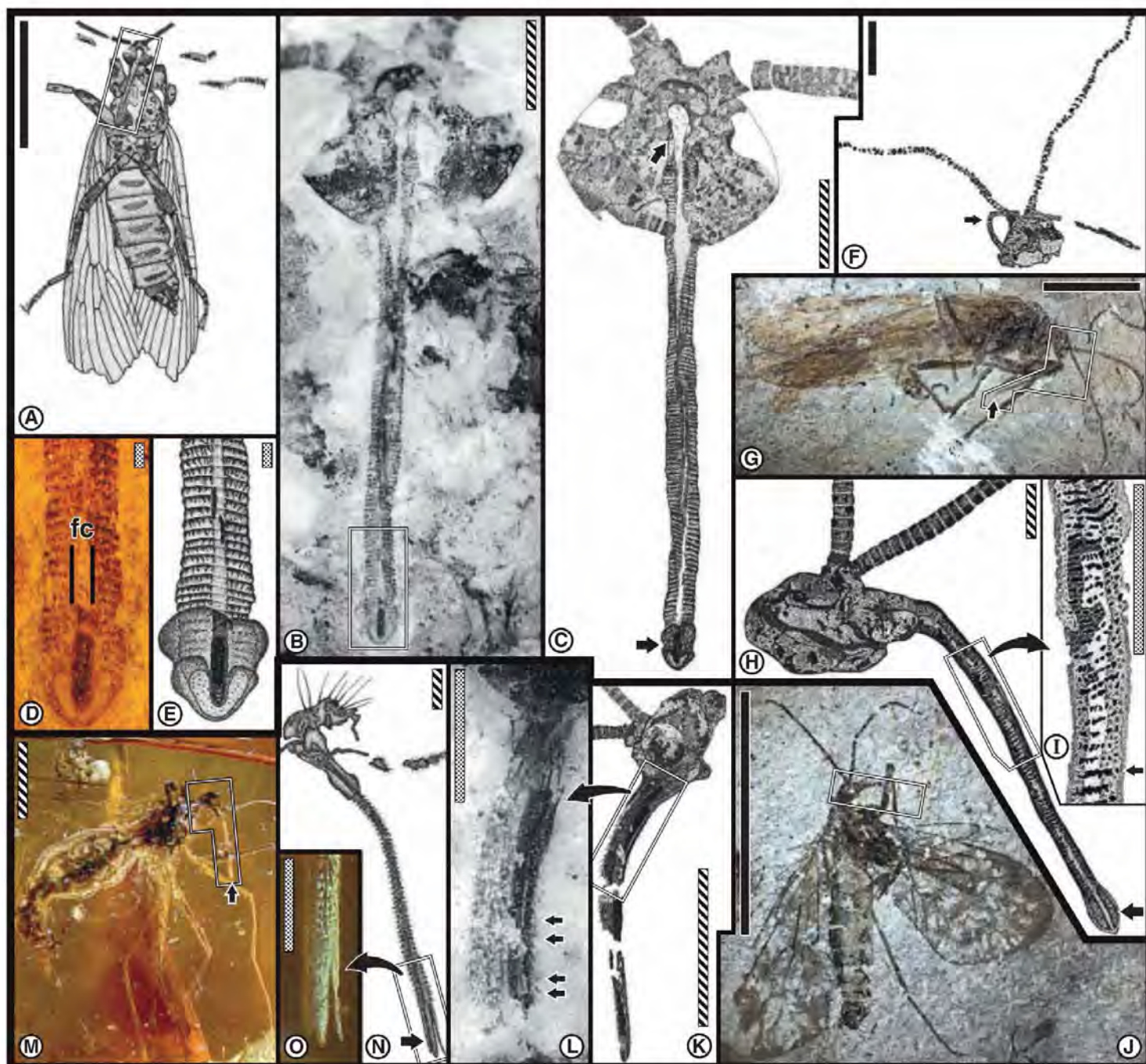


Fig. 2. Head and mouthpart features associated with fluid-feeding in two specimens of siphonate Aneuretropsychidae (A to E and G to I) and in three specimens of siphonate (F and J to L) and stylate (M to O) Pseudopolycenotropodidae, scorpionflies from the Middle Jurassic to late Early Cretaceous, from northeastern China and northern Myanmar (Burma). Shown in (A) to (E) is the head and mouthpart structure of *Jeholopsyche liaoningensis* Ren, Shih & Labandeira gen et sp. nov. (13), illustrating ventral body aspect (A) and dorsal view of head and mouthparts in photo (B) and drawing (C) (CNU-M-LB2005002). Note the filiiform antennae, palp absence, compound eyes surrounded by prominent ocular sclerite (ring), and bracketing of a prominent clypeal region housing a cibarial pump. The long siphon has thickened striate walls basally [(B), upper arrow in (C)] and is annulated by small transverse ridges, bearing a food canal (fc) and terminus with a V-shaped pseudolabellum (type 2), elongate-ellipsoidal mouth with opaque contents, and lateral fleshy lobes [lower arrow in (C)] in the photo (D) and drawing (E). A specimen (CNU-M-NN2005003) of *Pseudopolycenotropus janeannae* Ren, Shih & Labandeira sp. nov. (13) in (F), exhibiting a siphon curving 160° without fracture (arrow). (G) Another specimen (CNU-M-LB2005001) of an undetermined palpsless species of *Jeholopsyche*

shown in lateral view bearing a proboscis (arrow), probably in life position. (H) Enlargement of head and mouthparts boxed in (G), illustrating a robust proboscis base housing a cibarial pump and a terminus expanded to form a type 3 pseudolabellum (at arrow), homologous to that of (D) and (E). (I) Siphonal area boxed in (H) is enlarged to show segmented transverse ridges and food canal. Shown in (J) to (L) is the small species *Pseudopolycenotropus janeannae* Ren, Shih & Labandeira sp. nov. (13) (CNU-M-NN2005004), illustrated in right lateral view in (J) and left head profile of counterpart in (K). In (K) are broad circular eyes with diminutive ocular sclerite, absence of palps, well defined labrum (base of arrow), an externally smooth, finely pilose, external siphonal surface, and absence of any lobe-like modification of the rounded terminus. Dense, fine setae on the proboscis are enlarged in (L) (arrows). Shown in (M) to (O) is a specimen of *Parapolycenotropus burmiticus* (AMNH-Bu-1444) from Burmese amber. Entire insect (M) bears head and mouthparts, emphasizing stylate mouthparts within a labial tube. (N) Drawing [enlargement of boxed area in (M)] shows robust setae emerging from proboscis annulae and a distinct stylet, the terminus housing separated type 4 pseudolabellae [magnified in (O)]. Scale bars: solid, 10 mm; striped, 1 mm; dotted, 0.1 mm.

another plug of dark material in the mouth at the proboscis tip (fig. S2); the first two of these regions were controls. As expected, the matrix was highly depleted in carbon relative to the scorpionfly, but the relative abundance of carbon in the legs and proboscis plugs was

indistinguishable. One major result was the absence of iron enrichment in the food canal and mouth plugs relative to the leg or matrix. Sulfur exhibited a slight elevation in the insect relative to the matrix, and was uninformative regarding the presence of S-bearing biomole-

cules or their degradative by-products in the food tube or mouth. EDS data support a diet lacking in blood, suggesting that the food consisted of pollination droplets (1, 8, 26), and are noncommittal regarding the presence of any S-bearing amino acids in the food canal (13).

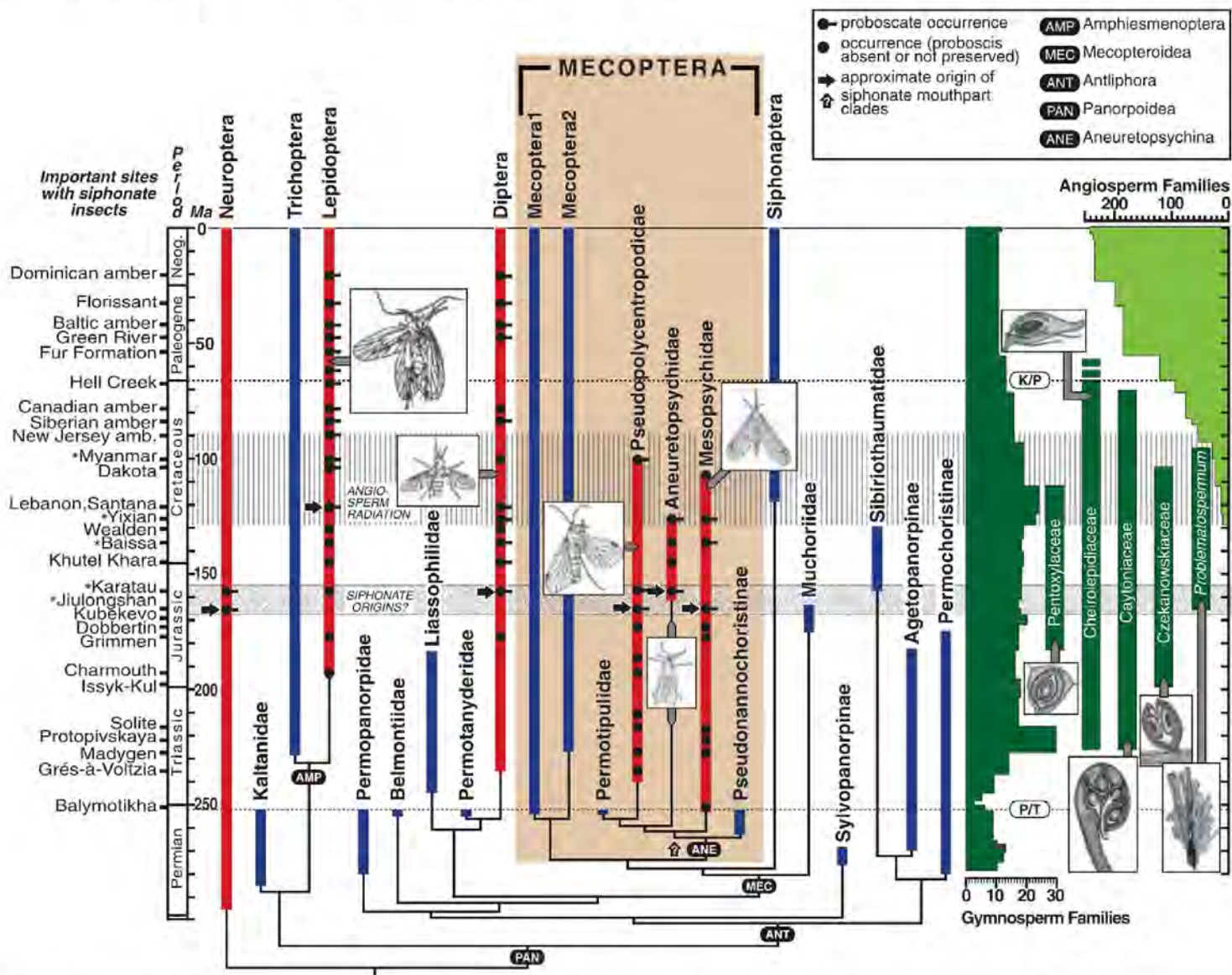


Fig. 3. Major clades of panoroid insects with siphonate mouthparts and their phylogenetic relationships (center) and likely associations with gymnospermous seed plants (right), presented in geochronologic context (left). At left are geologic periods calibrated to a time scale in millions of years (43); major Mesozoic localities are at the far left; asterisks denote fossil deposits in this report. Major extinction events are the Permian-Triassic (P/T) and Cretaceous-Paleogene (K/P) boundaries, indicated by the plant-insect associational record. At the center are broad, phylogenetic relationships derived from an analysis (13) of a morphology-based character matrix (tables S3 to S5) using a heuristic search in PAUP resulting in one of two best trees, shown here (length = 121, consistency index = 0.521, retention index = 0.725, rescaled consistency index = 0.378, homoplasy index = 0.479, and Goloboff fit criterion = -14.900) (fig. S1). Note the Mesozoic clade of long-proboscid Mecoptera (ANE) basal to remaining Mecoptera. All extant taxa and almost all geochronologically recent, nonsiphonate extinct taxa cluster into Mecoptera 1 ([Robinjohniidae + Bittacidae] + [Nannochoristidae + Boreidae]) and Mecoptera 2 (Panorpidae sensu stricto + [Dinopanorpidae + {Parachoristidae + Eomeropidae + (Meropeidae + Thaumatomeropidae)}]) clades. Alternative hypotheses for the origin of long-proboscid Mecoptera are presented as (i) basally within the clade during the Late Permian (bottom arrow

with "?"), or (ii) convergently in three constituent lineages during a ~13-My window in the mid-Jurassic, indicated by a stippled horizontal bar. The latter hypothesis is complemented by penecontemporaneous appearances of siphonate proboscides in a neuropteran and multiple brachyceran fly lineages (24), minimally representing five independent origins, indicated by vertical red bars and five accompanying arrows. By contrast, monophyletic siphonate Lepidoptera are delayed, paralleling the ecological rise of angiosperms. At right are gymnosperm (dark green) and angiosperm (light green) seed-plant diversity patterns (not to the same scale), compiled at conventional family levels (5, 44–50). Dark green vertical bars delineate known ranges of five gymnospermous clades indicated by cross sections of ovulate organs that could represent pollination mutualisms contemporaneous with long-proboscid scorpionflies (table S6). Illustrated are *Caytonia sewardi* Harris of Caytoniaceae (33, 51, 52), *Alvinia bohemia* Kvaček of Cheirolepidiaceae (8, 27), *Leptostrobus cancer* Harris of Czekanowskaceae (53), *Carnoconites compactus* Srivastava of Pentoxylaceae (36, 54), and gnetalean *Prolematospermum ovale* Turutanova-Ketova (28, 55). The transverse hatched bar within the Cretaceous (40 My deep) is the global transition from gymnosperm- to angiosperm-dominated floras; the topmost margin indicates the earliest angiosperm's tubular flowers with nectar (5).

Several gymnospermous plant hosts are present in the same or contemporaneous Eurasian deposits and could have been food sources for long-proboscid insects (27, 28). Coexisting seed-fern and conifer taxa secreted pollination droplets with surface-exposed fluid connected to deeper-seated pollination chambers by long tubules, enclosed channels, or analogous features (table S6). Species of Caytoniaceae, Cheirolepidiaceae, Czekanowskiaceae, Pentoxylaceae, and Gnetales (Fig. 3 and table S6) bore ovulate organs that were either unusual for wind pollination or possessed structures that would be expected for long-proboscid fluid feeding. Another possibility is that the immediate ancestors of angiosperms had incomplete carpel closure, with access to probing insects of deeper-seated pollination fluids. However, angiosperm taxa with floral features amenable to pollination by long-proboscid scorpionflies, such as tubular flowers or nectarial spurs, appear later in the fossil record (5, 8).

Insect proboscis dimensions from examined siphonate taxa could have been accommodated by micropylar or other connecting ovular tubule diameters. The data reveal three size patterns specific to each of the three insect clades (tables S2 and S6). Most scorpionfly taxa examined had food canal dimensions that could not accommodate pollen diameters of the inferred host-plant taxa (tables S2 and S6); this finding suggests that the mechanism for pollen transfer was bodily surface transport on mouthpart and head surfaces, analogous to most bee flies and hover flies (1). The first group were mesopsychids bearing elongate proboscides ~8.8 to ~10.1 mm long, whose relatively modest widths could accommodate the pollen catchment funnel (~7.0 mm long) of the ovulate organ in the cheirolepidiacean conifer *Alvinia bohémica*. The pollen catchment funnel of *A. bohémica*, lined by uniseriate trichomes distally and short, thickened glands proximally, was connected to a tube that traversed ovulate tissue to terminate immediately below the micropylar terminus adjacent to the cone axis (8, 27). The gnetalean *Problematospermum ovale* (28) potentially allowed access to pollination fluids through a long, tubular, exerted micropyle up to 12.0 mm long that was surrounded by a bracteate envelope of finely veined, petaloid structures. This feeding mode apparently was duplicated by coexisting, long-proboscid brachyceran flies (8, 29), analogous to extant nemognathine blister beetles that have proboscis lengths of ~10.0 mm (30), and by bee flies (31) and mydid flies such as *Rhaphiomidas* (32) having approximately the same proboscis lengths, pollinating deep-throated flowers. In the second group, Aneuretopsychidae, various species collectively possessed shorter siphons, 4.7 to 7.3 mm long, consistent with consumption of fluids produced by taxa such as *Caytonia* in tubules 4.0 to 6.5 mm long and 0.35 to 0.5 mm in diameter (33). Species of *Leptostrobus* (Czekanowskiaceae) also may have been a target, through which 2.5 to 5.0 mm

of similar distance would have been traversed for access to micropylar fluid opposite the entry area. Modern taxa bearing tubular proboscides of this length range include the tabanid fly *Mesopangonius* (proboscis length ~4.5 mm) (34) and the long-tongued vespid wasp *Ceramius hispanicus* (~5.6 mm) (35). The third group, diminutive species of Pseudopolycentropodidae, supported proboscides ranging from 0.9 to 1.8 mm in length, with diameters sufficiently small to enable them to target plants with equally short micropylar lengths. Plant host candidates include *Carnoconites compactus* (Pentoxylaceae) with extended micropylar-integumental lengths of 1.0 to 2.0 mm (36), overlapping with *Leptostrobus*, and conceivably surface accessible ovules of some bennettitalean lineages. Such short proboscides occur in several related species of nectar-feeding tabanids in the tribe Pangoniini (37), ranging in length from 0.6 to 1.7 mm, which lack stouter mouthparts that would characterize blood feeding.

Our case for insect pollination is based on a variety of evidence for these three lineages of scorpionflies and their inferred pollinated plants. First, scorpionfly proboscis morphology and surface features, such as hairy and obliquely ridged surfaces (Figs. 1 and 2), are highly convergent with extant dipteran, blister beetle (19, 30), and glossate lepidopteran mouthparts engaged in active pollination (1, 18, 19, 21, 31, 35). Second, co-occurring with these scorpionflies are seed-fern and conifer ovulate organs (table S6), which present pollen-receptive areas that are hidden or poorly exposed to wind-dispersed grains and lead into long integumental channels, prolonged catchment funnels, or extended tubular micropyles, ending in deeper-seated pollen chambers. Third, the levels of amino acids, sugars, inorganic ions, and other constituents found in modern insect-pollinated gymnosperms (26) suggest that the pollination droplets that filled these elongate tubular structures would be nutritionally similar to angiosperm nectar, thereby sustaining high levels of insect activity (8). Fourth, unisexual pollen-bearing organs typically are separated some distance from the conspecific ovulate organs, either on the same plant or different individual plants, thus favoring outcrossing for more efficient modes of pollination, such as remote insect transport. This contrasts with the bisexual system of known bennettitaleans and magnoliid angiosperms, in which female and male organs are adjacent to each other (38). Fifth, pollen extracted from affiliated pollen organs range in length from 17 to 65 μ m for the diverse ovulate taxa that we implicate for pollination (table S6), approximating the modern 25- to 50- μ m range for entomophilous cycads (39) and within the broader range of 10 to 80 μ m for similarly pollinated gnetaleans (40). The pollen of *Caytonia sewardi* possesses bisaccate pollen typically not associated with entomophily [but see (41)]; however, its compact pollen was within the entomophilous size range. Last, cheirolepidia-

ceous and gnetalean pollen occur as gut contents in several Mesozoic insect lineages (7, 8), serving as a nutritional source and/or as a reward for pollination.

Two hypotheses may explain the timing for the origin of the mecopteran siphonate proboscis from proposed nannochoistid-like ancestors: (i) The siphon was established during the Late Permian, for which there is phylogenetic but no fossil evidence (15, 16). (ii) Alternatively, if we consider only fossil data, three separate origins occurred in a ~13-My interval during the mid-Jurassic that also parallel the origin of dipteran and neuropteran siphons (Fig. 3). The latter hypothesis would indicate minimally a fivefold convergence among coeval aneuretopsychine scorpionfly and two other unrelated siphon-bearing lineages. The ~62-My subsequent phase of fluid-feeding on gymnospermous seed plants, and their probable pollination, eventually was replaced by a newly evolved, lepidopteran, siphon-bearing clade, Glossata, during the Early Cretaceous, probably as specialists on early angiosperm hosts (3, 42). Contemporaneously, this origin was accompanied by host-switching of siphonate, brachyceran fly lineages onto angiosperms and extinction of three siphonate lineages of scorpionflies.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/326/5954/840/DC1

Materials and Methods

SOM Text

Figs. S1 to S4

Tables S1 to S6

References

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Polymorphic Butterfly Reveals the Missing Link in Ecological Speciation

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Ecological speciation occurs when ecologically based, divergent selection causes the evolution of reproductive isolation. There are many empirical examples of this process; however, there exists a poorly characterized stage during which the traits that distinguish species ecologically and reproductively segregate in a single population. By using a combination of genetic mapping, mate-choice experiments, field observations, and population genetics, we studied a butterfly population with a mimetic wing color polymorphism and found that the butterflies exhibited partial, color-based, assortative mate preference. These traits represent the divergent, ecologically based signal and preference components of sexual isolation that usually distinguish incipient and sibling species. The association between behavior and recognition trait in a single population may enhance the probability of speciation and provides an example of the missing link between an interbreeding population and isolated species.

Research that has focused on a variety of biological systems has yielded compelling examples of ecological speciation (table S1) (1). Some of the specific traits that have diverged because of natural selection and cause reproductive isolation as a result include host choice in phytophagous insects such as *Rhagoletis* flies (2) and *Timema* walking sticks (3), body size in stickleback fish (4), coloration in cichlid fish (5) and poison-dart frogs (6, 7), and flowering time (8) and types of pollinators (9) in plants. Previous work

on ecological speciation has been instrumental in characterizing the direct link between natural selection and speciation (10, 11), but that work was largely focused on systems in which populations are highly differentiated (table S1). However, the means by which interbreeding populations transition to assortative mating on the basis of a trait that is under divergent natural selection are generally unknown.

Mimetic wing patterns in *Heliconius* butterflies provide a clear example of a trait involved in ecological speciation (12). *Heliconius* butterflies are chemically defended and warningly colored. Their evolutionary history has been marked by widespread divergence of color patterns among closely related species and geographic subpopulations (13). This divergence is combined with convergence among distantly related species as a result of natural selection for Müllerian mimicry (13), which is mimicry among

mutually protected species. Furthermore, closely related species and geographic subpopulations that differ in mimetic wing patterns generally exhibit color pattern-based assortative mate preference, whereby males preferentially approach and court females that share their color pattern (14, 15). Hence, selection for mimicry may generate premating reproductive isolation and precipitate speciation. For example, *H. cydno galanthus* and *H. pacheus* in Costa Rica are closely related, interfertile species that have different wing color patterns as a result of divergent natural selection to match different mimicry models, *H. sapho* and *H. hewitsoni* (Fig. 1A). DNA sequence data support the relative order of diversification, with the models being five times more divergent at mitochondrial DNA (mtDNA) than their mimics (16). The shift in mimicry between *H. cydno* and *H. pacheus* involves both color and pattern (Fig. 1B) and is accompanied by assortative mating (fig. S1), which is mediated by male preference for white versus yellow wing color (14, 17). Because hybrids have rarely been collected (18, 19), it appears that this assortative mate preference prevents substantial hybridization where the two species meet (Fig. 1A).

In western Ecuador, *H. cydno alithea* is locally polymorphic for the same white/yellow shift that generates premating isolation between *H. cydno galanthus* and *H. pacheus* in Costa Rica. As in Costa Rica, the color shift in *H. cydno alithea* appears to be caused by selection for mimicry, with the yellow morph matching *H. eleuchia* and the white morph matching *H. sapho* (Fig. 1A). Again, DNA sequence data support the relative order of diversification (16). Furthermore, field observations have demonstrated that *alithea* morph frequencies track those of *H. sapho* and *H. eleuchia* over time and space (20), and transplant experiments have demon-

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strated that the fitness of *alithea* morphs is determined by the local abundance of the models (20).

H. cydno alithea is polymorphic for both color (white versus yellow) and pattern (presence versus absence of melanin patches, Fig. 1A). The genetic basis of this color and pattern variation was determined by crosses (16), which revealed that color is controlled by a single Mendelian locus with a dominant white allele and a recessive yellow allele. Pattern is controlled by a second, unlinked Mendelian locus, with the presence of melanin dominant to the absence of melanin. *H. cydno galanthus* and *H. pachinus* from Costa Rica differ at five major color pat-

tern loci, two of which have virtually identical phenotypes to those seen segregating in *alithea*. The *K* locus, which is tightly linked to the gene *wingless* (14), controls white versus yellow, whereas the unlinked *Ac* locus (21) controls the presence versus absence of melanin in the same region of the forewing. Backcrosses between yellow *alithea* and white *galanthus* showed tight linkage between color and *wingless* [logarithm of the odds (lod) score = 11.0], just as in crosses between *galanthus* and *pachinus* (lod score = 18.2).

Assortative mate preference between *H. cydno galanthus* and *H. pachinus* is mediated by the

K locus in that males recognize conspecific females based on color (14, 17). Furthermore, male preference for white versus yellow is physically linked to the *K* locus (14). To determine whether there is color-based mate preference in *H. cydno alithea*, we tested 36 wild-caught and 139 captive-reared males for their preference for white or yellow *alithea* females (16). In total, we observed 1644 courtships by 115 males (Fig. 2). White and yellow males had divergent courtship preferences (likelihood-ratio test, $G_{11} = 35.18$, $P = 2.32 \times 10^{-4}$), with yellow males exhibiting a pronounced preference for yellow females (Wald's test, $Z = 6.143$, $P < 10^{-9}$). White males had no

Fig. 1. Parallel divergence and Müllerian mimicry in *Heliconius* butterflies. (A) In Costa Rica, co-mimetic species pairs are restricted to opposite drainages. In western Ecuador, *H. eleuchia*, *H. sapho*, and polymorphic *H. cydno alithea* (red arrows point to alternate *Ac* phenotypes) co-occur. (B) Spectral reflectance measurements of light (white or yellow) and dark (black or iridescent blue) wing patches show concordance between co-mimics and parallel divergence in Costa Rica and Ecuador (averaged across 10-nm intervals; error bars are standard deviation).

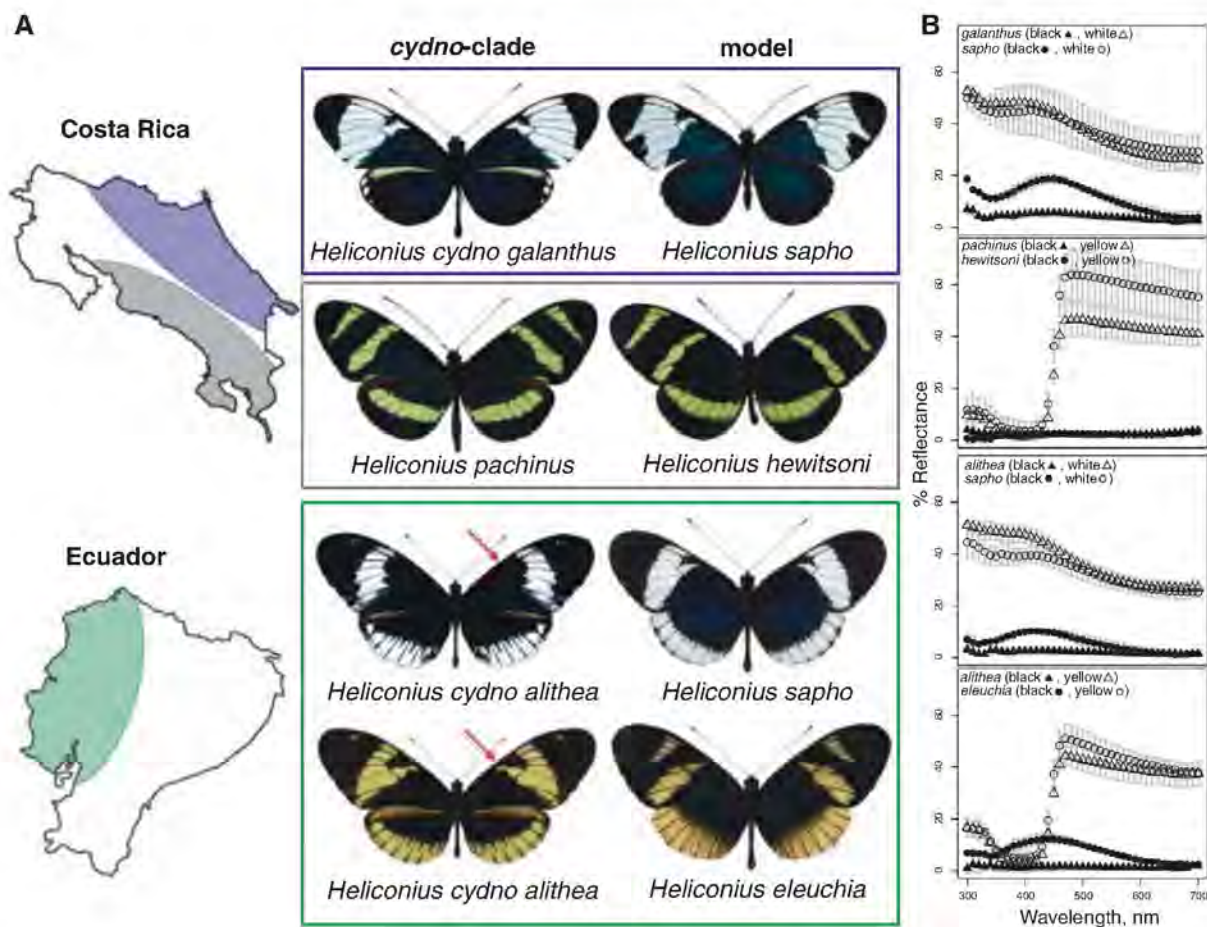
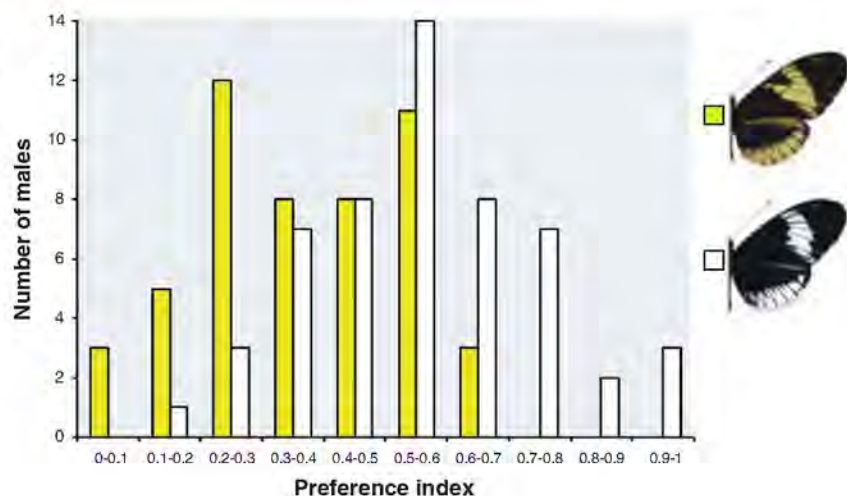


Fig. 2. Distribution of *H. cydno alithea* mate preference indices for yellow [yellow bars, maximum likelihood estimate = 0.36 (0.423 to 0.299)] and white [white bars, maximum likelihood estimate = 0.54 (0.604 to 0.475)] males. The preference index (x axis) is the proportion of courtship and attempted mating events that were directed toward white females; a preference index of 1 indicates complete preference for white, whereas 0 indicates complete preference for yellow. All males with two or more courtship events are shown.



significant preference ($Z = 1.142$, $P = 0.254$). Male preference was also affected by pattern differences: Yellow females with the melanic *Ac* patch were more attractive than yellow females without it ($G_{12} = 27.24$, $P = 7.14 \times 10^{-3}$). Male *Ac* phenotype and status (wild versus captive) had no effect on preference. Field observations were also consistent with our experimental results, showing no color bias in white males' chasing and mating behavior ($G_1 = 0.97$, $P = 0.33$) but significant bias in yellow males' behavior toward yellow females ($G_1 = 10.98$, $P = 9 \times 10^{-4}$).

The difference in mate preference between the *H. cydno alithea* morphs may be explained if the color forms actually represent partially isolated subspecies with overlapping distributions, much like sympatric host races in phytophagous insects. Thus, we genotyped white and yellow *alithea* specimens at over 800 amplified fragment length polymorphisms (AFLPs), sequenced 1600 base pairs of mtDNA, and generated comparative data for *H. cydno galanthus*, *H. pacheus*, and three outgroup species: *H. melpomene*, *H. atthis*, and *H. ismenius*. Phylogenetic and population genetic analyses showed that closely related *alithea*, *galanthus*, and *pacheus* were differentiated (Fig. 3), but there was no genetic differentiation between white and yellow *alithea*: analysis of molecular variance (AMOVA)-based $F_{ST} = 0.001$ ($P = 0.35$) for AFLPs and $F_{ST} = 0.057$ ($P = 0.10$) for mtDNA. Furthermore, clustering of the AFLP data with

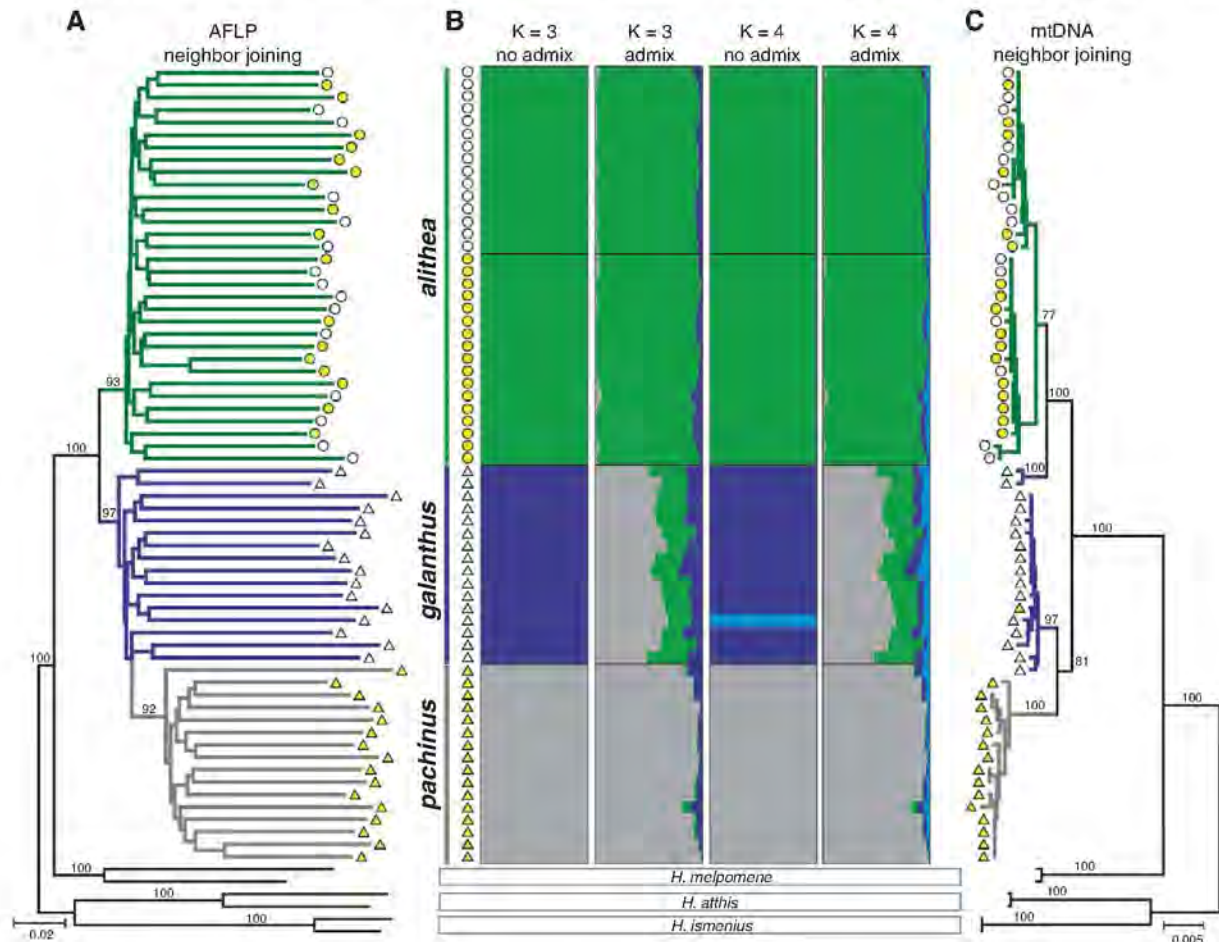
the program STRUCTURE (22) failed to detect any color-associated subdivision in *alithea* (Fig. 3B), and constraining the morphs to form separate clusters resulted in a very poor fit to the data [difference in $\ln(\text{likelihood}) = -319$]. We also tested this hypothesis by examining patterns of linkage disequilibrium (LD) between the unlinked color-patterning loci *K* and *Ac* (16). Strong assortative mating between the morphs should generate LD among unlinked markers, yet we found no association between *K* and *Ac* in 68 wild-caught butterflies ($P = 1.00$). These results, combined with frequent observations of mixed mating in greenhouse cultures, indicate that the *alithea* morphs are not reproductively isolated and hence appear to represent a single polymorphic interbreeding population.

Another mechanism that could explain mimicry-based mate preference in *alithea* is genetic linkage between color and preference. Male color preference co-segregates with alleles at the color locus *K* in crosses between *H. cydno galanthus* and *H. pacheus*, and first-generation hybrids have intermediate preference (14). Our *alithea* data support this hypothesis, because yellow males ($K^Y K^Y$, where Y indicates yellow allele) exhibited yellow preference, but white males, the majority ($\approx 83\%$) of which were likely to be heterozygous at *K* ($K^W K^Y$, where W indicates white allele), did not. We hypothesize that this genetic association involves a mechanism that prevents recombination, such as a

single locus controlling both traits or an inversion, because otherwise, interbreeding between the morphs would disassociate them over time. Single-locus control of signal and preference is unlikely, generally (23), but may be facilitated in this case because ommochrome pigments color nymphalid butterfly wings (24) and serve as lateral filtering pigments in insect eyes (25). Lastly, the association between color and preference in *alithea* is not likely to be due to self-referent phenotype matching, whereby males recognize their own color and preferentially court females that display the same color, because this would result in a white-biased preference among white males, similar to the yellow bias observed in yellow males.

H. cydno alithea may represent a very early stage in the evolution of reproductive isolation, when the same traits that distinguish sister species, in this case mimicry and color-based mate preference, segregate in a single population (supporting online material). To examine the relationship between *alithea* and other examples of ecological speciation, we reviewed the literature and scored 20 relevant biological systems for criteria that define the progression of speciation (table S1). A total of 10 criteria were summarized as the following five categorical variables (table S2): number of divergent phenotypic traits, genetic basis of these traits, presence versus absence of postzygotic reproductive isolation, degree of spatial segregation, and strength of genetic dif-

Fig. 3. Population genetics of *H. cydno alithea*, *H. cydno galanthus*, and *H. pacheus*. (A) Bootstrap neighbor-joining tree of polymorphic AFLP markers (bootstrap values > 50% are shown). (B) STRUCTURE clustering (no-admixture and admixture) on AFLP data correctly identified the three populations at $K = 3$, but clustering at $K = 4$ resulted in a reduced likelihood and did not subdivide *alithea* by color. (C) Bootstrap neighbor-joining tree of mtDNA sequences (bootstrap values > 50% are shown). In each panel, individuals are designated by colored symbols (triangles for the Costa Rican taxa *H. cydno galanthus* and *H. pacheus*, circles for *H. cydno alithea*; white or yellow indicates wing color).



ferentiation. Multiple correspondence analysis of these variables revealed that *H. cydno alithea* differed from other examples of ecological speciation in that divergence is based on a single-locus trait and is not accompanied by postzygotic isolation or background genetic differentiation (fig. S2). Research on other examples of ecological speciation has revealed populations that may also be in an early stage of divergence (26–28), suggesting that continued examination of these and other systems will reveal a continuum in the trajectory of ecological speciation, as is evident in *Heliconius* butterflies.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/326/5954/847/DC1

Materials and Methods

SOM Text

Figs. S1 and S2

Tables S1 and S2

References

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A Type I–Secreted, Sulfated Peptide Triggers XA21-Mediated Innate Immunity

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The rice *Xa21* gene confers immunity to most strains of the bacterium *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). Liquid chromatography–tandem mass spectrometry analysis of biologically active fractions from *Xoo* supernatants led to the identification of a 194–amino acid protein designated Ax21 (activator of XA21-mediated immunity). A sulfated, 17–amino acid synthetic peptide (axY²²) derived from the N-terminal region of Ax21 is sufficient for activity, whereas peptides lacking tyrosine sulfation are biologically inactive. Using coimmunoprecipitation, we found that XA21 is required for axY²² binding and recognition. axY²² is 100% conserved in all analyzed *Xanthomonas* species, confirming that Ax21 is a pathogen-associated molecular pattern and that XA21 is a pattern recognition receptor.

In 1995 we showed that the rice *Xa21* resistance gene, which encodes a protein with predicted leucine-rich repeat (LRR), transmembrane, juxtamembrane, and intracellular kinase domains, conferred immunity to diverse strains of the Gram-negative bacterium *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) (1, 2). Subsequent discoveries in flies (3), humans (4), mice (5), and *Arabidopsis* (6, 7) revealed that animals and other plant species also carry membrane-anchored receptors [Toll in flies; Toll-like receptor 4 (TLR4) in mice and humans] with striking structural similarities to XA21 and that these receptors are also involved in microbial recognition and defense. Like XA21, these receptors typically associate with or carry non-RD

(non-Arg-Asp) kinases to control early events of innate immunity signaling (8). *Arabidopsis* FLS2 (flagellin-sensitive 2) and EFR (elongation factor receptor) belong to the same class of plant receptor kinases (the LRRXII) as XA21 (8, 9).

Many of these cell surface receptors were later named pattern recognition receptors (PRRs) on the basis of their ability to directly recognize molecules that are conserved across a large class of microbes (10, 11). Such microbial molecules were called pathogen-associated molecular patterns [PAMPs, also known as microbe-associated molecular patterns (MAMPs)] (12).

Despite the similarity of the known PRRs to XA21, the classification of XA21 has been debated (13, 14). This is partly because XA21 was discovered before the terms PRR and PAMP were established (12) and partly because, under the classical definition of Flor (15), XA21 was called a “resistance” gene. Furthermore, because the molecule recognized by XA21 [previously called avrXa21 (avirulence Xa21) and here renamed

Ax21 (activator of XA21-mediated immunity)] had not been identified, it was not known whether this molecule was conserved among a large class of microbes—a hallmark of PAMPs (12).

We previously identified six *Xoo* genes required for ax21 activity (the *rax* genes), which fall into two functional classes. The first class consists of three genes (*raxA*, *raxB*, and *raxC*) that encode components of a bacterial type I secretion system (TOSS) (16). The second class is involved in sulfation, including *raxST*, which encodes a protein with similarity to mammalian tyrosine sulfotransferases (16). *Xoo* strains carrying mutations in any of these *rax* genes no longer activate XA21-mediated immunity. None of the identified genes encodes an obvious activator of immunity.

To identify Ax21, we fractionated the supernatant of *Xoo* strain PXO99 cultures on a C18 reversed-phase high-performance liquid chromatography (RP-HPLC) column (Fig. 1A) and carried out bioassays of seven HPLC peptide-enriched fractions (Fig. 1B) using our previously established methods (17). An active fraction that was able to trigger XA21-mediated immunity (Fig. 1) was subjected to liquid chromatography–tandem mass spectrometry (LC-MS/MS) (18). Fifteen peptides from the LC-MS/MS spectra matched eight *Xoo* proteins (18), including two peptides that corresponded to the N-terminal and C-terminal regions of a 194–amino acid protein encoded by PXO_03968 (boxes in Fig. 1C and fig. S1).

To identify which gene encodes Ax21, we generated *Xoo* strains carrying a mutation in each of the individual genes. Whereas a PXO_03968 knockout strain caused long lesions and grew to high levels on XA21 leaves (Fig. 2), none of the other strains did (fig. S2). These data indicate that the PXO_03968 gene encodes Ax21. We further showed that Ax21 secretion requires *raxA* and *raxC* (fig. S3) (18).

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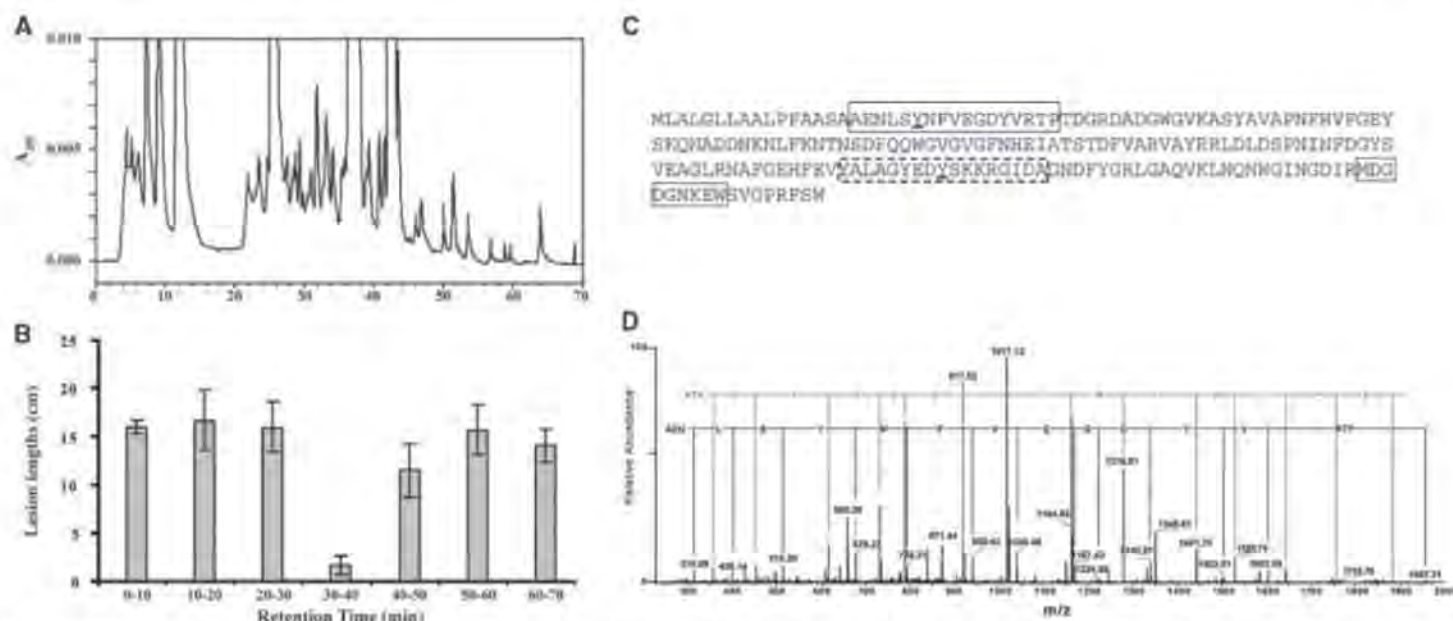


Fig. 1. Isolation of Ax21. **(A)** RP-HPLC elution profile of peptides secreted from *Xoo* strain PXO99 (carrying Ax21 activity). Peptide-enriched samples from the PXO99 supernatant were separated on a reversed-phase C18 column (1 × 250 mm, flow rate 0.05 ml/min) with a 10 to 90% acetonitrile gradient containing 0.1% trifluoroacetic acid. A_{280} , absorbance at 280 nm. **(B)** Lesion length measurements of XA21 rice leaves pretreated with RP-HPLC fractions followed by inoculation with PXO99 Δ raxST. Lesion lengths were measured 12 days after PXO99 Δ raxST inoculation. Each value is the mean $\pm$ SD from nine

inoculated leaves. **(C)** Deduced amino acid sequence of Ax21. The two peptides (boxed) identified from the biologically active fraction were sequenced using LC-MS/MS. Predicted sulfated tyrosines Y22 and Y144 are underlined. The dashed box indicates one of the peptide used in the Ax21 bioassay shown in Fig. 3. **(D)** Mass (LTQ) spectrum of the axY22 peptide corresponding to the N-terminal region [first box in (C)] of Ax21. The spectrum corresponding to the peptide derived from the C-terminal region [second box in (C)] of Ax21 is shown in fig. S1.

To test the importance of the putative tyrosine sulfation sites on Ax21 (19), we synthesized seven peptides: two carrying sulfated tyrosines in the target residues [Tyr²² and Tyr¹⁴⁴ (Y22 and Y144)], two carrying nonsulfated tyrosines, two carrying alanines in place of the tyrosines, and one corresponding to the C-terminal region of Ax21 (Fig. 3A) (19). XA21 rice leaves were pretreated with each peptide (100 μ M in water). The 17-amino acid peptide carrying Y22 sulfation (axY^{S22}) activated XA21-mediated immunity (Fig. 3B). To further quantify this response, we characterized the activity of the axY^{S22} synthetic peptide by growth curve analysis. Pretreatment of XA21 rice leaves with the axY^{S22} peptide triggered resistance to PXO99 Δ raxST, as reflected in a reduction in PXO99 Δ raxST population growth by three orders of magnitude. The nonsulfated peptide (axY22) was unable to trigger XA21-mediated immunity (Fig. 3C).

Bioassays with 17 axY^{S22} peptide variants carrying alanine substitutions identified eight amino acids critical for XA21-mediated immunity (fig. S4A) (18). A concentration of 1 μ M is sufficient for PAMP activity (fig. S4B) (18).

In coimmunoprecipitation experiments with hemagglutinin (HA)-tagged axY^{S22} and extracts from leaves carrying a Myc-tagged XA21 protein (18), we observed labeling of a band migrating at 140 kD by SDS-polyacrylamide gel electrophoresis (PAGE) with antibodies to both Myc and HA (Fig. 4). The presence of 5- to 10-fold excess untagged axY^{S22} peptide suppressed the labeling of this band, whereas flg22_{ave} from

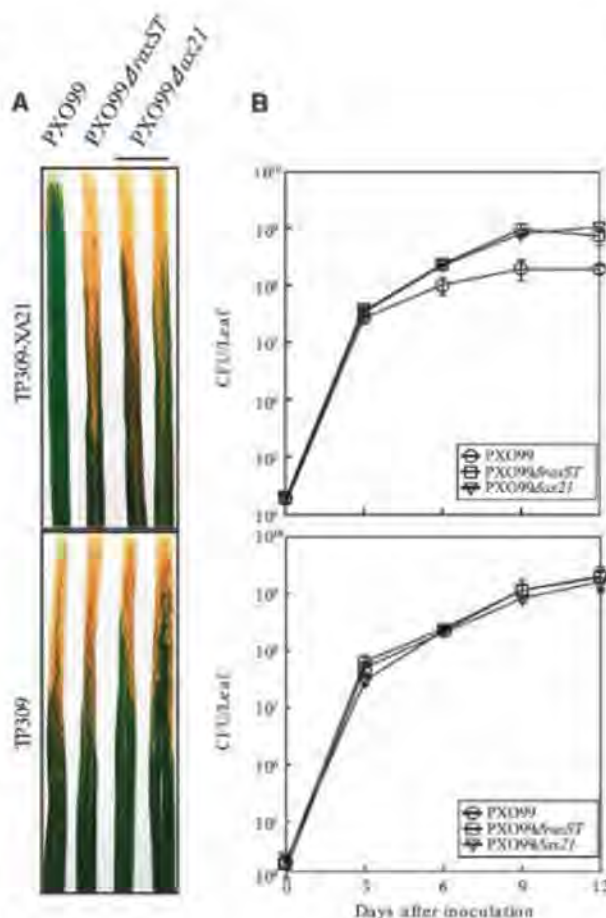


Fig. 2. A mutation in *ax21* abolishes Ax21 activity. **(A)** Lesion lengths of rice leaves measured 12 days after inoculation with *Xoo* strains PXO99, PXO99 Δ raxST, or PXO99 Δ ax21. Suspensions of each strain [10^8 colony-forming units (CFU)/ml] were scissor-inoculated onto rice leaves (TP309-XA21, resistant to PXO99; TP309, susceptible to PXO99). Images are representative of five independent experiments. **(B)** Growth of PXO99, PXO99 Δ raxST, and PXO99 Δ ax21 populations in inoculated rice leaves. Bacteria were extracted from the leaves at 0, 3, 6, 9, and 12 days after inoculation, plated on selective media after serial dilution, and colonies counted after a 3-day incubation at 28°C. Each value is the mean $\pm$ SD from nine inoculated leaves.

the rice pathogen *Acidovorax avenae* had no effect on axY^{S22}-XA21 binding (Fig. 4). These experiments demonstrate that XA21 is required for axY^{S22} binding and recognition.

Sequence analysis indicates that Ax21 is highly conserved in *Xoo* strains (KACC 10331 and MAFF 311018, both 98% identity), *X. campestris* pv. *campestris* (90%), *X. axonopodis* pv. *glycinea* (92%), *X. axonopodis* pv. *vesicatoria* 85-10 (*Xav*) (92%), and *X. oryzae* pv. *oryzicola* (98%) (fig. S5).

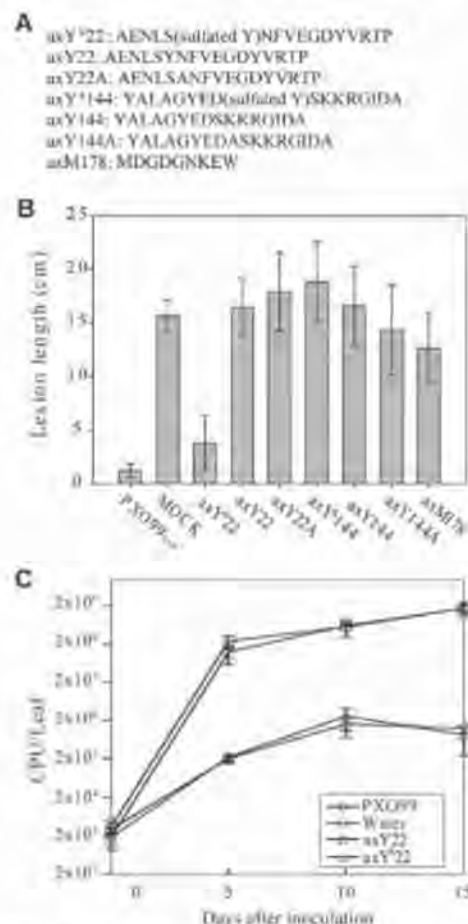


Fig. 3. The axY^{S22} peptide is sufficient to trigger XA21-mediated immunity. **(A)** Synthetic peptides, including three corresponding to the N-terminal region of AX21 (axY^{S22}, axY^{S22A}, and axY^{S22B}), three corresponding to the central region (axY^{S144}, axY^{S144A}, and axY^{S144B}), and one corresponding to the C-terminal region (axM178), were tested for activity. Abbreviations for amino acid residues: A, Ala; D, Asp; E, Glu; F, Phe; G, Gly; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; Y, Tyr. **(B)** Five hours after peptide pretreatment, leaves were inoculated with PXO99Δ*raxST* and the lesions measured 12 days later. Each value is the mean $\pm$ SD from six leaves. **(C)** Growth of PXO99Δ*raxST* populations over time. TP309-XA21 leaves were pretreated with PXO99 supernatant (PXO99_{sup}), water, or the synthetic peptides (axY^{S22} and axY^{S22A}, 100 μ M each). Bacterial cells were extracted from the leaves at 0, 5, 10, and 15 days after inoculation, plated on selective media after serial dilution, and colonies counted after a 3-day incubation at 28°C. Each value is the mean $\pm$ SD from eight inoculated leaves.

S5). The 17-amino acid axY^{S22} sequence is 100% conserved in these strains. *Xylella fastidiosa* and the opportunistic human pathogen *Stenotrophomonas maltophilia* also carry putative Ax21 orthologs (48% and 61%, respectively) and show 77% and 65% similarity, respectively, to the axY^{S22} sequence (fig. S5).

Because *Xav* carries predicted orthologs for Ax21, *raxST*, *raxA*, and *raxB*, which we have previously shown to be required for Ax21 activity (16), we hypothesized that *Xav* would express Ax21 activity. Indeed, we found that pretreatment of XA21 rice leaves with supernatants from wild-type *Xav*, but not *Xav* strains carrying a deletion of *ax21* (18), can activate XA21-mediated immunity (fig. S6). These results indicate that the *ax21* ortholog in *Xav* possesses the predicted biological activity.

One of the key aspects of the definition of PAMPs is that they “are conserved within a class of microbes” (10). As a result of the explosion of studies on PRRs and PAMPs in both plant and animal systems, it has now become clear that PAMPs can be conserved quite widely across genera (e.g., flagellin) or more narrowly within a genus (e.g., Pep13) (20) and, further, that sequence variation and posttranslational modifications can modulate PRR-dependent pathogen recognition (18, 21).

In the XA21-Ax21 system, the axY^{S22} peptide sequence is invariant in all sequenced *Xanthomonas* species. Sulfation provides specificity to the system, just as flagellin or lipopolysaccharide recognition in some hosts is modulated by glycosylation or acylation, respectively (21, 22). Thus, Ax21 is a PAMP that satisfies the genetic definition of an avirulence factor because the presence or absence of sulfation on the conserved 17-amino acid epitope is decisive for its ability to trigger XA21-mediated

immunity. Similarly, *Xa21* is a disease resistance gene because it is the single polymorphic determinant in rice that confers resistance to strains of bacteria expressing sulfated Ax21, and it is also a PRR because it is required for recognition of a particular modified peptide epitope that is conserved across a microbial genus.

Thus, our data provide another example of bacteria-host interactions that can be attributed to the presence of genes encoding proteins (e.g., sulfotransferases, glycosylases, and acetylases) that modify conserved peptide epitopes (21–25). Such examples indicate that successful pathogens of plants and animals have evolved methods of altering the PAMP to avoid detection by the host PRR. Conversely, the presence or absence of a particular PRR can have a marked effect on the resistance of the host to infection. Just as plants deficient in XA21 or FLS2 exhibit reduced resistance to phytopathogens, mice deficient for TLR4 or TLR2 are altered in their response to *Mycobacterium tuberculosis* infection (22). These studies have led to a convergence in our understanding of the molecular mechanisms governing the specificity of host-microbe interactions in plants and animals.

Our results thus demonstrate that the definitions of PAMPs and *avr* genes and those of disease resistance genes and PRRs cannot be strictly separated. Future usage of these terms should reflect the concepts presented by Medzhitov (12) and Flor (15), and must also take into account subsequent discoveries of the effects of PAMP polymorphism and posttranslational modifications. In plants, a disease resistance gene is merely an allele in the host genotype that confers resistance in a particular interaction. Such genes can encode diverse proteins (although, to date, the great majority encode intracellular nucleotide-binding LRR

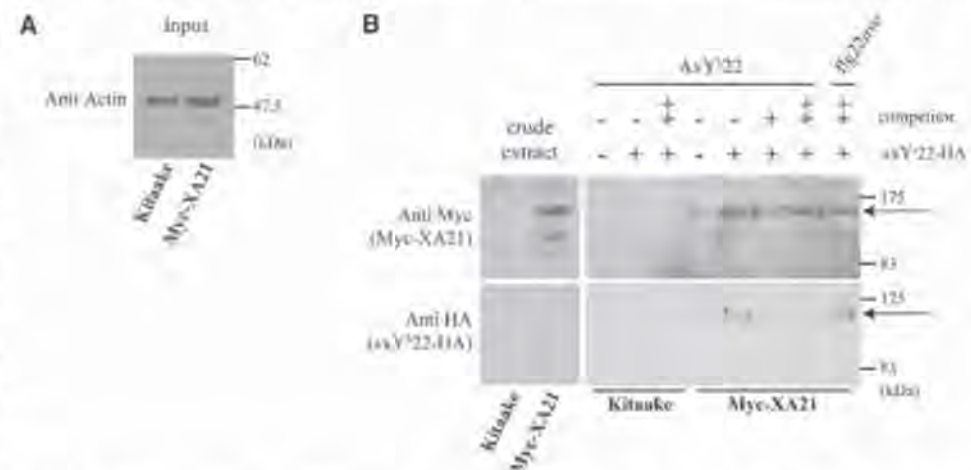


Fig. 4. XA21 is required for axY^{S22} binding. HA-tagged axY^{S22} cross-links to a 140-kD polypeptide that is immunoprecipitated by an antibody to Myc (Myc-XA21). **(A)** Before immunoprecipitation, the loading of equal amounts of protein (50 μ g) from Kitaake and Myc-XA21 leaf extracts was confirmed using an antibody to actin (input). **(B)** Leaf extracts were incubated with 1 mM HA-axY^{S22} in the presence (+, 5 mM; ++, 10 mM) or absence (–) of the competitors axY^{S22} lacking the HA tag or flg22_{ave}. After binding, cross-linking was initiated by the addition of sulfo-Ethylene Glycol bis (Succinimidyl Succinate). Duplicate protein gels were analyzed after separation by SDS-PAGE using antibodies to Myc (top) and to HA (bottom). Myc-XA21 and a proteolytic cleavage product of Myc-XA21 were detected at 140 and 110 kD, respectively, as reported previously (32). Arrows indicate the XA21 and Ax21-XA21 complexes.

proteins) (2, 26–28). Hence, the term “*R* gene” is merely operational and mechanistically agnostic.

Likewise, the term “avirulence gene” remains useful as a broad term that indicates a gene that encodes any determinant of the specificity of the interaction with the host. Thus, this term can encompass some PAMPs and pathogen effectors (e.g., bacterial type III effectors and oomycete effectors) as well as any genes that control variation in the activity of those molecules (29–31)].

In the future, a diverse array of PAMPs from plant pathogens will likely be discovered. Many of these will almost certainly serve as ligands for the large class of predicted orphan PRRs present in the genomes of plant species (371 in rice; 47 in *Arabidopsis*) (8).

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Materials and Methods

Figs. S1 to S6

Tables S1 and S2

References

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Small-Molecule Activators of a Proenzyme

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Virtually all of the 560 human proteases are stored as inactive proenzymes and are strictly regulated. We report the identification and characterization of the first small molecules that directly activate proenzymes, the apoptotic procaspases-3 and -6. It is surprising that these compounds induce autoproteolytic activation by stabilizing a conformation that is both more active and more susceptible to intermolecular proteolysis. These procaspase activators bypass the normal upstream proapoptotic signaling cascades and induce rapid apoptosis in a variety of cell lines. Systematic biochemical and biophysical analyses identified a cluster of mutations in procaspase-3 that resist small-molecule activation both in vitro and in cells. Compounds that induce gain of function are rare, and the activators reported here will enable direct control of the executioner caspases in apoptosis and in cellular differentiation. More generally, these studies presage the discovery of other proenzyme activators to explore fundamental processes of proenzyme activation and their fate-determining roles in biology.

Activation of proteases triggers a myriad of biological events, such as apoptosis and blood clotting, both inside and outside of the cell (1). Proteases are generally stored as inactive proenzymes that are usually activated by upstream proteases or by themselves. These activation events may sometimes involve binding a protein partner or, in rare instances, interaction with a natural small molecule (2) or peptide (3). In the case of autoproteolysis, the proenzyme must achieve not only an active state, but also one in which the sites of proteolysis

are exposed. In situ activation of specific pro-proteases with synthetic small molecules could uncover new molecular principles in zymogen activation and would facilitate direct control of these important processes in biology.

The executioner procaspases, consisting of procaspases-3, -6 and -7, represent excellent initial candidates for discovery of small-molecule protease activators. Caspases are a family of homodimeric cysteine proteases responsible for many of the fate-determining processes in cell biology, including apoptosis, innate immune signaling, early stages of stem cell differentiation, and cellular remodeling (4–6). As with most proteases, caspases are synthesized as inactive procaspases, or zymogens, and are activated by upstream proteolysis or autoproteolysis. Previous studies have shown that the mature active caspases are intrinsically

dynamic (7, 8) and sample both an “on state” and an “off state” that structurally resemble the zymogen-like conformation (9, 10). Small molecules have been found to trap these two forms of the mature enzyme (11, 12). We reasoned that if the procaspases existed in a similar dynamic equilibrium of off and on states, it might be possible to find small molecules that promote autoproteolytic activation via stabilization of an on-state conformation. Executioner procaspases are particularly good targets as they are susceptible to rapid activation by both upstream proteases and self-proteolysis. Thus, any activation would be accentuated in trans by autocatalytic activation. Moreover, these particular caspases are essential for executing the final processes of apoptosis, and specific activation by a small molecule would elicit robust and precise phenotypic responses within cells.

High-throughput screening was employed to identify compounds that could promote autoproteolytic activation of procaspase-3 at physiological concentrations [for HTS design, see (13)]. A dozen compounds out of 62,000 promoted >20-fold activation of procaspase-3 (fig. S1) and were resynthesized to validate their chemical composition. To warrant further analysis, dynamic light-scattering was used to select compounds with solubilities greater than 100 μ M, and a β -lactamase inhibition assay was performed to discard promiscuous aggregators (14). The most robust procaspase activator fulfilling these criteria was compound 1541, a substituted phenylimidazopyridine-methoxy coumarin with a median effective concentration (EC_{50}) for activation of 2.4 μ M (Fig. 1A). Mass spectrometry revealed that the compound did not covalently label either the mature or proenzyme forms of caspase-3, nor was 1541 modified by enzyme hydrolysis.

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We compared the rate of 1541 activation and specificity for the executioner procaspases-3, -6, and -7, which share 40 to 50% sequence identity. Granzyme B, a natural proteolytic activator of procaspase-3, rapidly and fully activates the zymogen within 90 min. In contrast, after an initial slow phase that lasts ~30 min, 1541 induces accelerated activation of caspase-3 to a level that is finally ~70% that of granzyme B within 2.5 hours (Fig. 1B). Granzyme B and 1541 both induce full proteolytic processing of procaspase-3, as evidenced by production of the large (17-kD) and small (12-kD) subunits of caspase-3 (fig. S2). Procaspase-7 is robustly activated and fully processed by granzyme B, but not by 1541 (Fig. 1C). The compound 1541 does induce ~10% self-cleavage of its prodomain (residues 1 to 23) (fig. S2), but prodomain removal by itself does not elicit caspase-7 activation (15). Procaspase-6 is resistant to granzyme B (16, 17), but 1541 promotes activation and complete self-processing similar to procaspase-3 (Fig. 2D) (18). Furthermore, 1541 does not activate or induce self-cleavage of inflammatory procaspase-1 (fig. S3) (19). We also tested PAC-1, a compound previously reported to induce a slight activation of procaspase-3 (20), but found no detectable increase in activity or self-proteolysis among the executioner procaspases (Fig. 1, B to D). Others have reported a similar inability to activate procaspase-3 with PAC-1 (21–24). Thus, 1541 is a highly specific and robust activator of executioner procaspases-3 and -6, and does not activate procaspases-1 or -7.

To further validate activity and specificity, several 1541 analogs with modifications on the coumarin ring were tested (Fig. 1A, and fig. S4). Substitution of the 8-methoxy with an 8-hydroxy (1541B) improves the EC_{50} by about twofold for activation of procaspase-3, but dramatically reduces activity toward procaspase-6. A 6-bromo substitution (1541C) reduces potency for procaspase-3 and for procaspase-6 by factors of 15 and 35, respectively. Deletion of the imidazopyridine substituent meta to the coumarin on the phenyl ring (1541D) eliminates activation of procaspases-3 and -6 (Fig. 1A). Thus, relatively modest modifications to 1541 can alter efficacy and selectivity, which suggests that 1541 has high specificity to the site of interaction.

A series of *in vitro* biochemical and kinetic analyses was performed to assess the activation mechanism of procaspase-3 by 1541 and to test our initial hypothesis of autoproteolysis through stabilization of an on-state conformation (Fig. 1E). Mature caspase-3 has a catalytic efficiency (k_{cat}/K_M) of $2.5 \text{ min}^{-1}\mu\text{M}^{-1}$ for hydrolyzing a fluorogenic peptide substrate Ac-IETD-AFC that mimics the activating cleavage site (residues 172 to 175) between the large and small subunits (Fig. 2A). Procaspase-3 is substantially less active (by a factor of 2100), with a corresponding k_{cat}/K_M of $0.0012 \text{ min}^{-1}\mu\text{M}^{-1}$. We titrated procaspase-3 with compound 1541B (from 0 to 50 μM) and measured the kinetic parameters for Ac-IETD-AFC by Michaelis-Menten analysis within 15 min,

where no detectable processing or self-activation of procaspase-3 occurred that would contribute to substrate recognition and cleavage. As 1541B is increased, a dose-dependent decrease is observed in K_M (101 to 14 μM , a factor of seven) and an increase in k_{cat} (0.12 to 0.93 min^{-1} , a factor of eight) (Fig. 2A). Compound 1541B, therefore, induces an immediate kinetic state where the catalytic efficiency is increased 57-fold (k_{cat}/K_M of $0.068 \text{ min}^{-1}\mu\text{M}^{-1}$) over unstimulated procaspase-3. Note that the K_M value for procaspase-3 improves to 14 μM in the presence of 1541B, compared with 212 μM and 101 μM for the

mature enzyme and unstimulated proenzyme, respectively. These data strongly imply that the initial catalytic activity of 1541B-stimulated procaspase-3 results from a different on-state conformation than that of mature caspase-3 and represents the first intermediate of the activator-bound procaspase-3 (Fig. 1E). For hydrolyzing amide substrates, K_M is a reasonable estimate of the K_D (25, 26). Thus, the 1541B-activated procaspase-3 binds Ac-IETD-AFC about 15 times as tightly as the mature enzyme, albeit with a k_{cat} value substantially lower than that of the mature enzyme (Fig. 2A) (27). Small amounts of mature

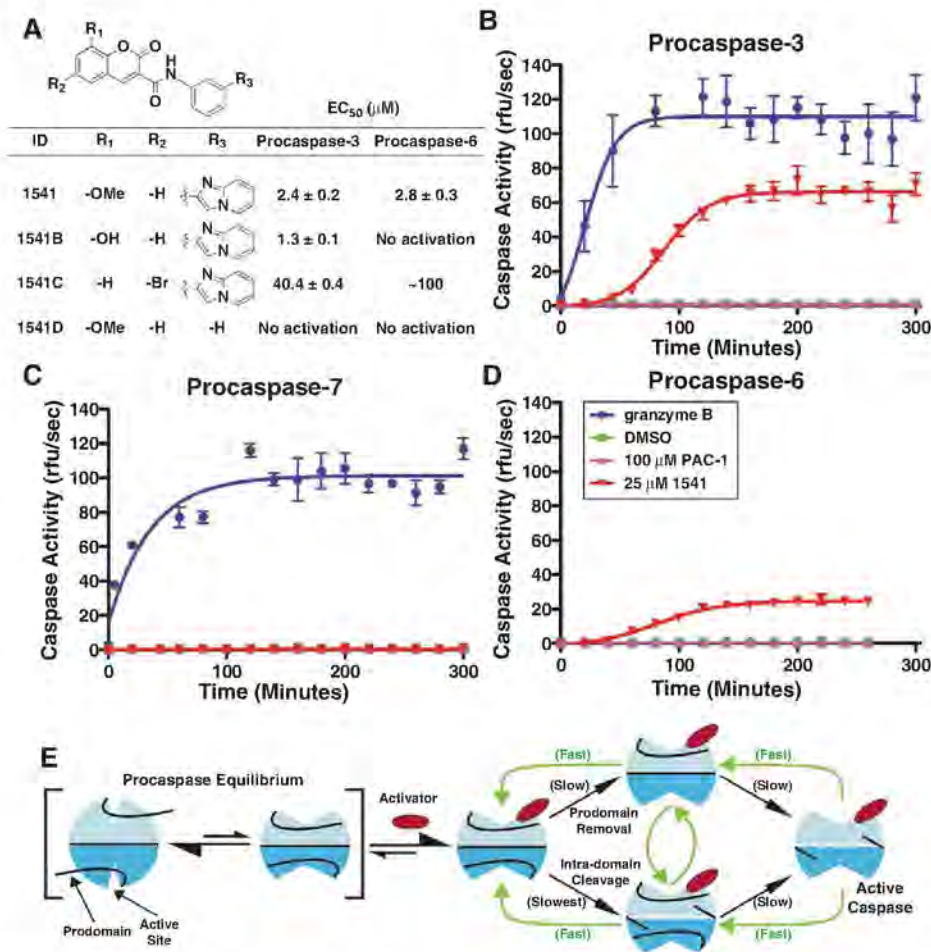


Fig. 1. Specificity of small-molecule activators for executioner procaspases. (A) Chemical analogs of 1541 and EC_{50} values of procaspases-3 and -6 activation. Small-molecule activators require the imidazopyridine moiety and prefer a heteroatom substituent at the 8-position on the coumarin ring (42, 43). Substitution of the 8-methoxy with an 8-hydroxy (1541B) improves the EC_{50} and specificity for activation of procaspase-3 over procaspase-6. Addition of a 6-bromo (1541C) reduces potency of activation of both procaspases. Complete removal of the imidazopyridine substituent (1541D) ablates activation of procaspases-3 and -6. (B to D) Time course of executioner procaspase activation by 1541 facilitates full self-cleavage of procaspases-3 and -6. Procaspases-3 (B), -7 (C), and -6 (D) were incubated at 100 nM with granzyme B, DMSO (green on baseline), PAC-1, or 1541 at 37°C and assayed every 20 min for activity by addition of fluorogenic peptide substrates Ac-DEVD-AFC (caspases-3 and -7) or Ac-VEID-AFC (caspase-6) (44). Rfu, relative fluorescence units. (E) Proposed model for small molecule-assisted procaspase self-activation. We hypothesize that procaspases are in a dynamic equilibrium between an off state (left) and on state (right), similar to mature caspases. Unlike mature caspases, the population favors the off-state conformation. On binding a small-molecule activator, the equilibrium shifts to an on state (center). This complex slowly undergoes autoproteolytic activation (black arrows) that accelerates with increasing production of mature caspase (green arrows). This model also accounts for activation, at low concentrations, of small molecule where the on state is preferred and one active site is available in the dimer for processing and, at high concentrations, where both sites are saturated and can lead to inhibition.

caspase-3 that are generated in the presence of 1541 eventually overtake the activation process and account for the subsequent rapid increase in activity.

As activation of caspase zymogens requires cleavage of the junction between the large and small subunits, we tested whether these compounds could also enhance the proteolytic susceptibility of the proenzyme itself. We generated an inactive procaspase-3 by mutating the catalytic cysteine, C163A (in which Cys¹⁶³ is replaced by Ala), and then tested whether 1541 enhances C163A processing by granzyme B (28). Incubation with granzyme B alone resulted in ~10% cleavage of C163A after 30 min and 20% after 60 min. Addition of 1541 significantly increased the rate of processing to 40% after 30 min, and 80% after 60 min (Fig. 2B). Coincubation with a nonactivating analog 1541D (Fig. 1A) did not enhance proteolysis by granzyme B. Thus, these activating compounds increase both the catalytic efficiency of unprocessed procaspase-3 (Fig. 2A) and its susceptibility to proteolysis (Fig. 2B). The ability to induce autoproteolytic activation was

then evaluated by titrating 1541 (0.1 to 100 μ M) with wild-type procaspase-3 and measuring activation as a function of time (Fig. 2C). As the incubation times increase, the EC_{50} values of the activation curves shift to lower values, and the slopes dramatically increase, which are indicative of a highly cooperative activation process consistent with the feedback activation proposed in Fig. 1E (29).

We next investigated why the maximal autoproteolytic activation of procaspase-3 was only about 70% of that induced by granzyme B (Fig. 1B), even though procaspase-3 was fully processed (fig. S2). Titration of procaspase-3 with 1541 (from 0.1 to 100 μ M) assayed after 4 hours shows a biphasic dose-response (Fig. 2D). At low concentrations, the enzyme is activated (EC_{50} of 2.4 μ M) whereas, at high concentrations, it is partially inhibited [median inhibitory concentration (IC_{50}) of 34 μ M]. The proenzyme becomes fully processed over the time course of the dose-response study (EC_{50} of ~2 μ M) (fig. S6). To understand the basis for this inhibition, we directly tested 1541 on mature caspase-3. Indeed,

caspase-3 is inhibited by 1541 with a virtually identical IC_{50} of 35 μ M (Fig. 2E). A similar biphasic dose-response curve was observed for procaspase-6 (EC_{50} of 2.8 μ M and IC_{50} of 38 μ M). Michaelis-Menten analyses revealed that 1541 acts as a competitive or mixed noncompetitive inhibitor of caspases-3 and -6 (fig. S7). Overall, these results are consistent with a mechanism whereby, at low concentration, a single 1541 molecule binds near one active site of the dimer as an inhibitor and stabilizes an on-state conformation that promotes self-cleavage at the unoccupied subunit (Fig. 1E). At very high concentrations, 1541 can inhibit both active sites.

On the basis of kinetic inhibition data of caspase-3 by 1541 (fig. S7) and conservation with caspase-6, we used alanine-scanning mutagenesis on residues near the active site to probe for resistance mutations (Fig. 2F). Three procaspase-3 mutants (S198A, T199A, and S205A) (28) rendered the proenzyme insensitive to 1541 activation. Each of the three variants were efficiently activated by granzyme B, which showed that these were fully functional enzymes (Fig. 2G). These mutational

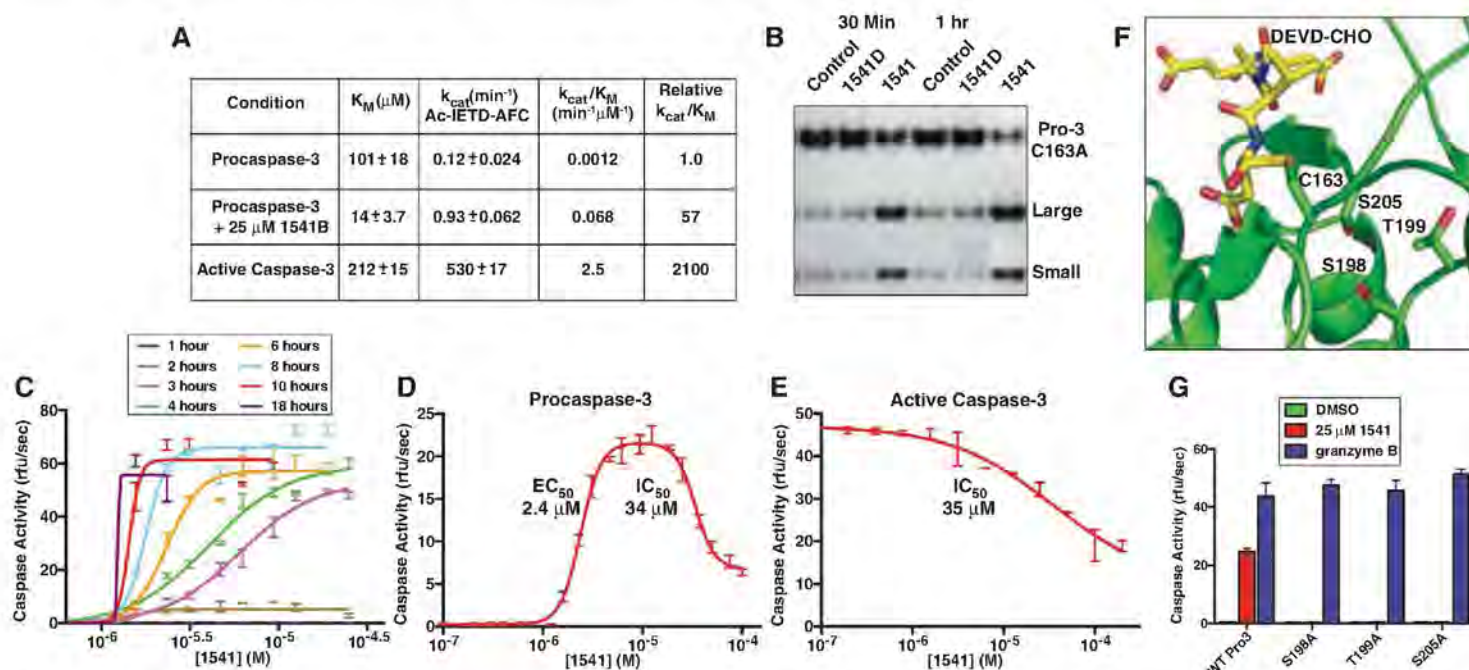


Fig. 2. In vitro characterization of apoptotic procaspase small-molecule activators. (A) Initial rates of activation of procaspase-3 in presence of 1541B, as measured by Ac-IETD-AFC compared with unstimulated zymogen and mature caspase-3. Procaspase-3 (100 nM) was incubated with 1541B (0.78 to 50 μ M), and initial rates of activity were measured over ~15 min by addition of Ac-IETD-AFC (1.2 to 300 μ M) at 37°C. Presence of 1541 improves the k_{cat}/K_M of procaspase-3 by 57-fold and stimulates autocatalytic processing. (B) Silver-stained SDS-polyacrylamide gel electrophoresis (SDS-PAGE) gel of inactive C163A procaspase-3 (250 nM) incubated with granzyme B (0.5 nM), with and without 1541 (25 μ M), shows increased cleavage susceptibility by granzyme B with 1541, but not with the inactive analog 1541D. (C) Procaspase-3 was incubated at 100 nM with 1541 (0.1 to 100 μ M) to assess the rate of self-activation. Kinetic activity of the mixtures was determined every hour for 18 hours by incubation with 20 μ M Ac-DEVD-AFC. Once procaspase-3 is activated by 1541, the zymogen rapidly self-activates. The effective concentration of 1541 is ~1.5 μ M, with no activation below this concentration despite extended incubation periods. (D) Procaspase-3 at 100 nM was incubated with 1541 (100

nM to 100 μ M). After a 4-hour incubation at 37°C, the samples were assayed for caspase activity by addition of Ac-DEVD-AFC. Low concentrations of 1541 induce activity of procaspase-3 with an EC_{50} of 2.4 μ M, and, at high concentrations, inhibit with an IC_{50} of 34.0 μ M. (E) Compound 1541 exerts a similar inhibitory effect on active caspase-3 (IC_{50} = 35 μ M), as seen for procaspase-3. Mature caspase-3 was incubated at 25 nM in various concentrations of 1541 for 10 min and assayed with Ac-DEVD-AFC. (F) Structural model of the active site of procaspase-3 showing the residues targeted for mutation. The location of the DEVD substrate is shown and modeled from caspase-3 in complex with tetrapeptide aldehyde inhibitor Ac-DEVD-CHO (Protein Data Bank structure 2DKO). (G) Identification of mutations in procaspase-3 that confer resistance to activation by 1541. Wild-type and mutant (S198A, T199A, and S205A) procaspases were incubated for 5 hours with 25 μ M 1541 (red), 1 nM granzyme B (blue), or DMSO (green on baseline) at 37°C and sampled for activation via kinetic assays initiated by addition of substrate Ac-DEVD-AFC. The procaspase-3 variants S198A, T199A, and S205A displayed wild-type activity when activated by granzyme B, but were unable to self-activate in the presence of 1541.

studies indicate specific residues near the active site that alter binding of 1541 and/or susceptibility to autoproteolysis of wild-type procaspase-3.

We next tested the ability of 1541 to induce apoptosis in a p53-deficient breast cancer cell line, BT549. Cells were treated with 1541 (25 μ M), as well as with traditional inducers, such as staurosporine (STS) [a nonspecific kinase inhibitor (30)] and etoposide [a topoisomerase II inhibitor (31–33)]. Cells were probed every two hours for cellular viability and executioner caspase activity (DEVDase) (28). After 4 hours, 1541 and STS induce comparable and near-complete apoptosis of BT549 cells (Fig. 3A). Etoposide induced much slower apoptosis because of dependence on p53 for activity. The decrease in cellular viability in presence of STS and 1541 is accompanied by a reciprocal increase in DEVDase activity, characteristic of caspase-3, which peaks after 8 hours (Fig. 3B). DEVDase activity is significantly higher with STS than with 1541. Western blots confirmed production of active caspases-3, -6, and -7 and the hallmark apoptotic cleavage of poly(adenosine diphosphate-ribose) polymerase (PARP); however, more extensive cleavage is observed with STS (fig. S8). Procaspase-7 is also cleaved in cells treated with 1541, which likely reflects the ability of mature caspases-3 and/or -6 to activate procaspase-7, as previously reported (15) (fig. S8).

Two other cell lines were tested: MDA-MB361, a p53-proficient breast cancer cell line; and HEK293, a transformed human embryonic kidney cell line (fig. S9). A similar pattern of cell viability and DEVDase activity was observed in MDA-MB361, as for BT549 cells. In HEK293 cells, 1541 was the most potent apoptotic inducer, compared with STS and etoposide, and generated the highest level of DEVDase activity. These data suggest that 1541 is as potent an inducer of cell death as STS and functions independently of p53. The fact

that STS and 1541 induce comparable apoptotic rates, despite the higher caspase activity associated with STS, suggests that activation of only a fraction of the pool of executioner procaspases in these cells is required to induce cell death. Compounds 1541C and D, respectively, induce limited to no apoptosis or DEVDase activity in these three cell lines (Fig. 3A and fig. S9). Significantly, the cellular results for these 1541 analogs correlate with their *in vitro* activity against procaspase-3 (Fig. 1A). Preincubation of cells with irreversible cell-permeable caspase inhibitors [carbobenzoxy-valyl-alanyl-aspartyl-(O-methyl)-fluoromethylketone (VAD-FMK) or carbobenzoxy-aspartyl-(O-methyl)-glutamyl-(O-methyl)-valyl-aspartyl-(O-methyl)-fluoromethylketone (DEVD-FMK)] completely blocked apoptosis with 1541, which indicated that the caspase activity is responsible for the observed apoptosis (Fig. 3C).

The breadth of 1541 induction of apoptosis was then assessed in a variety of cancerous and transformed cell lines including BT549, MDA-MB361, HEK293, HeLa, and HCC1954. Cells were incubated with increasing concentrations of 1541 and assayed for cell death after 24 hours (fig. S10). All cell lines underwent apoptosis, with EC₅₀ values ranging between 4 and 9 μ M, similar to those measured *in vitro* for procaspases-3 and -6 (EC₅₀ of 2 to 3 μ M). Compound 1541B, which is comparably active toward procaspase-3, but inactive against procaspase-6, exhibited virtually the same EC₅₀ for inducing apoptosis (fig. S10). These results imply that direct activation of procaspase-3 alone is sufficient to induce rapid apoptosis.

We assessed the effects of 1541-induced apoptosis on apoptotic hallmarks and signaling pathways. Typical inducers of the intrinsic apoptosis pathway, such as STS, operate indirectly to activate executioner procaspases by releasing mitochondrial cytochrome c into the cytoplasm, which

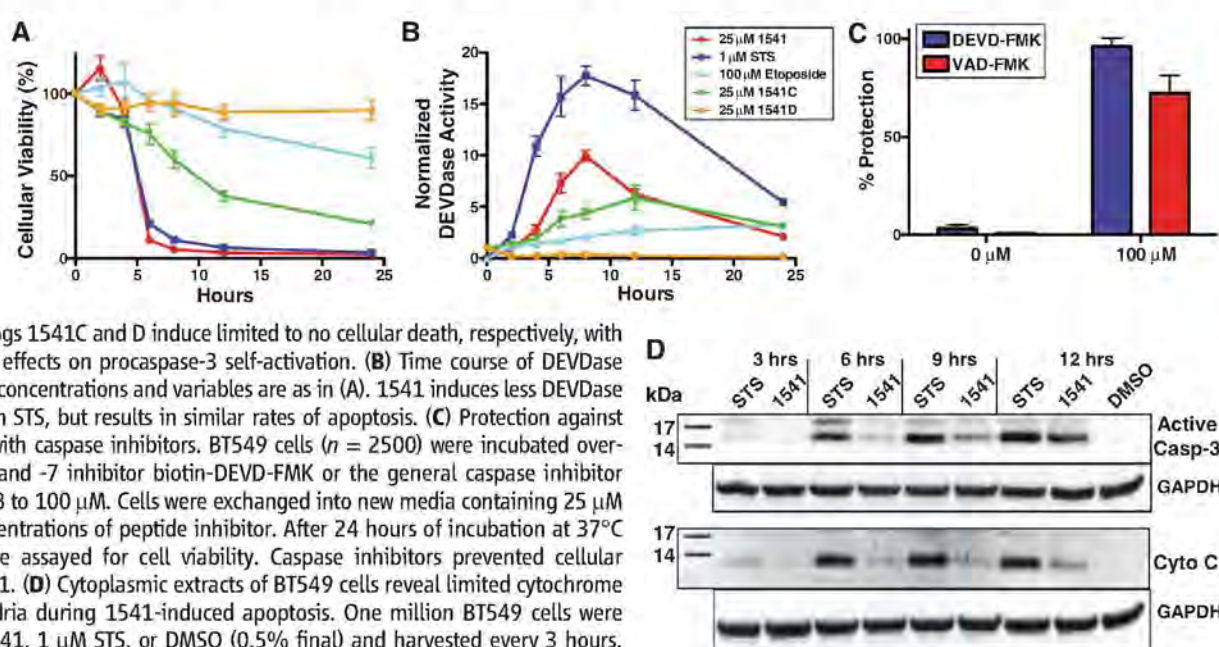
leads to activation of procaspase-9, which in turn targets executioner procaspases. Direct activation of procaspase-3 by 1541 should be independent of cytochrome c in the cytoplasm. BT549 cells were incubated with either 1541 or STS and harvested every 3 hours, and cytoplasmic extracts were probed by Western blot to establish levels of activated caspase-3 and cytochrome c (Fig. 3D). Indeed, STS induced much greater and more rapid mitochondrial cytochrome c release than did 1541. As expected, small amounts of cytochrome c appeared in the cytosol at later time points with 1541, because positive-feedback loops from activated caspase-3 are known to produce mitochondrial damage eventually, as well as subsequent cytochrome c release (34).

We next evaluated if 1541 induces apoptosis directly through the executioner caspases or if the compound requires an intact extrinsic or intrinsic cell death pathway. To test the requirement for the extrinsic cell death pathway, wild-type (A3) and caspase-8-deficient human Jurkat cells (I9.2) (35) were treated with 1541, STS, and FasL, a specific inducer of the extrinsic pathway (Fig. 4A). Compound 1541 produced rapid and comparable rates of apoptosis in both wild-type and caspase-8-deficient Jurkat cells. As expected, FasL was active in wild-type Jurkats, but not in caspase-8-deficient cells. STS induction was comparable to 1541 in wild-type Jurkats, and somewhat slower in caspase-8-deficient cells, which suggested that the extrinsic pathway augments some of its activity. Thus, cells with genetic lesions within either the intrinsic (p53) or extrinsic (caspase-8) pathways are resistant to traditional intrinsic or extrinsic inducers, respectively, but both are susceptible to cell death by 1541.

To determine whether 1541 targets are downstream of the mitochondria within the intrinsic pathway, we compared induction of apoptosis in

Fig. 3. Induction of apoptosis in a p53-deficient breast cancer cell line by 1541. (A) Time course of cell viability in BT549 cells. BT549 cells ($n = 2000$) were incubated with compounds as shown in the key or DMSO (final 0.5%) and assayed for cell viability at 2, 4, 6,

8, 12, and 24 hours. Analogs 1541C and D induce limited to no cellular death, respectively, with correlation to the *in vitro* effects on procaspase-3 self-activation. (B) Time course of DEVDase activity in BT549 cells. All concentrations and variables are as in (A). 1541 induces less DEVDase activity in comparison with STS, but results in similar rates of apoptosis. (C) Protection against 1541-induced apoptosis with caspase inhibitors. BT549 cells ($n = 2500$) were incubated overnight with the caspase-3 and -7 inhibitor biotin-DEVD-FMK or the general caspase inhibitor biotin-VAD-FMK from 0.78 to 100 μ M. Cells were exchanged into new media containing 25 μ M 1541 and respective concentrations of peptide inhibitor. After 24 hours of incubation at 37°C with 1541, the cells were assayed for cell viability. Caspase inhibitors prevented cellular apoptosis induced by 1541. (D) Cytoplasmic extracts of BT549 cells reveal limited cytochrome c release from mitochondria during 1541-induced apoptosis. One million BT549 cells were incubated with 25 μ M 1541, 1 μ M STS, or DMSO (0.5% final) and harvested every 3 hours. Cytoplasmic supernatants were subjected to Western blot analysis for presence of active caspase-3 or cytochrome c. Compared with STS, 1541 induced far less release of cytochrome c during apoptosis.



wild-type murine embryonic fibroblasts (MEFs) and MEFs that lack the proapoptotic proteins Bak and Bax (36–38). Deletion of Bak and Bax prevents release of mitochondrial cytochrome c and blocks the apoptotic response of intrinsic apoptotic inducers STS and etoposide. Wild-type MEFs and Bak^{-/-}/Bax^{-/-} double-knockout (DKO) MEFs were incubated with STS or 1541 and then assayed for apoptosis by using a histone enzyme-linked immunosorbent assay (ELISA) that measures solubilization of histones caused by DNA fragmentation, a hallmark of apoptosis (39). As expected, wild-type MEFs were susceptible to apoptosis by either 1541 or STS (Fig. 4B). However, DKO MEFs retained sensitivity to 1541, but were completely resistant to STS. Western blots probed for active caspase-3 and PARP confirmed cleavage of these markers in 1541-induced DKO MEF lysates, but not in STS-treated cells (Fig. 4C). Thus, 1541 can bypass the lesion of Bak and Bax, which is consistent with direct activation of procaspase-3.

To decide whether the compounds act specifically through procaspase-3 in cells, we tested the ability of 1541 to induce apoptosis in human

MCF-7 cells that lack a functional caspase-3 gene (40, 41). Indeed, MCF-7 cells were far less susceptible to 1541-induced apoptosis, which could be restored upon stable transfection with wild-type procaspase-3 (Fig. 4D). Cells transfected with the S198A procaspase-3 mutant, previously determined in vitro to resist 1541-induced activation, also largely resisted apoptosis in cells. This mutant did exhibit some apoptosis after a 24-hour incubation, which may arise from leaky activation of S198A procaspase-3. Overall, these data provide strong support that procaspase-3 is the primary intracellular target of 1541 and a major mediator of 1541-induced apoptosis in vivo. To begin to explore a therapeutic window for procaspase activators, we tested the effects of 1541B on a cancerous B cell line (DB) in comparison with Epstein-Barr virus transformed immortalized normal B cells (EBV). We found the cancerous cell line to be more sensitive to apoptosis induced by 1541B (fig. S11). Further experiments will be needed to explore these compounds as selective chemotherapeutic agents that bypass typical proapoptotic lesions.

In conclusion, our studies reveal a unique mechanism for activation of executioner procaspases by synthetic small molecules. Remarkably, the compounds both reorganize the active site for catalysis and render their internal cleavage sites more accessible to proteolysis, which may reflect aspects of the natural zymogen-activation process. These compounds could either stabilize the on state of procaspase-3 or destabilize the off state and enhance susceptibility to proteolysis through a more flexible on-state conformation. The small-molecule analogs exhibit selectivity toward procaspase-3 in vitro and in cells. Selective small-molecule activators of procaspases will have great utility for in-depth studies of caspase-dependent apoptotic and nonapoptotic processes, such as stem cell differentiation (6) and cellular remodeling (5). Finally, our results, together with the recently characterized allosteric activation of the monomeric *Vibrio cholerae* RTX cysteine protease by a natural small-molecule metabolite (2), suggest that it may be possible to discover synthetic small-molecule activators for other proenzymes to facilitate functional and mechanistic studies and further to enhance their utility in biology and disease.

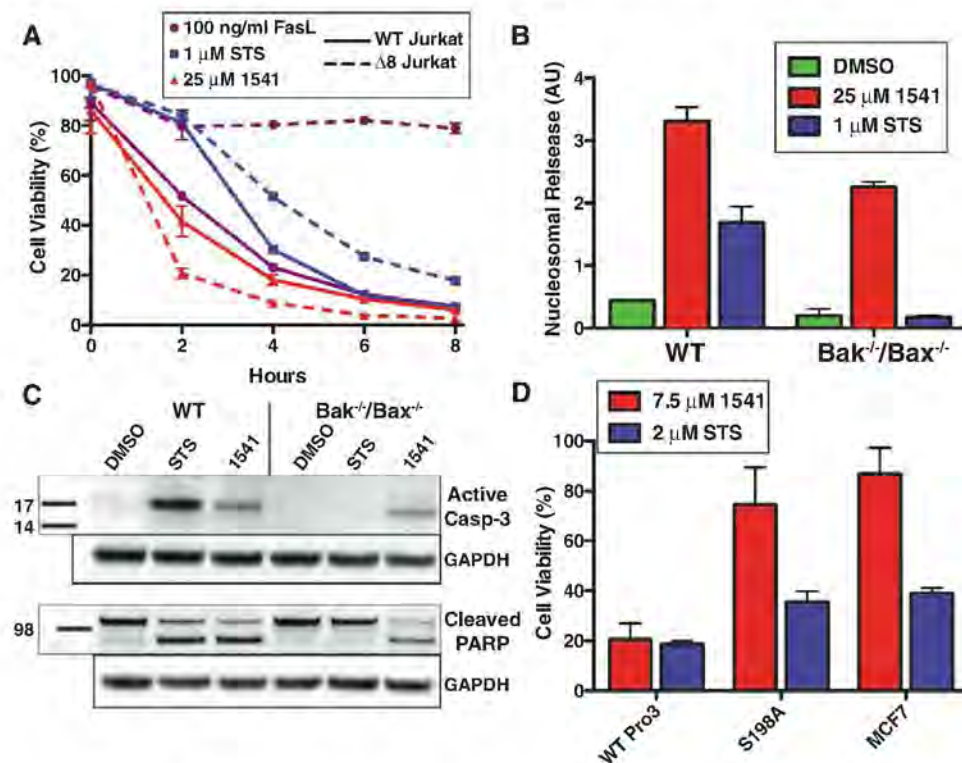


Fig. 4. Validation of executioner procaspases as cellular target of 1541. (A) Wild-type (A3) (solid line) or caspase-8-deficient (19.2) (dotted line) Jurkat cells (100,000 cells) were incubated with 1541, STS, or FasL and assayed for cellular viability every 2 hours. 1541 induces cell death independent of the extrinsic pathway. (B) Cells ($n = 5000$) derived from either wild-type or Bak^{-/-}/Bax^{-/-} DKO MEFs were incubated with STS, 1541, or DMSO (final 0.5%) at 37°C. A histone sandwich ELISA (Roche) was performed on the lysates to determine the extent of apoptosis after 12 hours. Induction of cell death by 1541, in the absence of Bak and Bax, supports direct activation of the executioner procaspases. (C) For Western blot analysis, one million cells of both wild-type and Bak^{-/-}/Bax^{-/-} DKO MEFs were plated and incubated as in (B) for 12 hours. Western blots of whole-cell lysates were probed for the presence of active caspase-3 and cleavage of PARP. (D) Transfection of MCF-7 cells with wild-type procaspase-3 and 1541-resistant S198A procaspase-3 supports ablation of 1541-induced procaspase-3 activation in cells. Cells ($n = 6000$) from stably transfected MCF-7 cell lines were incubated for 24 hours with 1541, STS, or DMSO (final concentration 0.5%) and assayed for cell viability. S198A procaspase-3 MCF-7 cells resisted apoptosis similarly to the parental MCF-7 cell line; wild-type procaspase-3 transfection of MCF-7 cells had increased apoptotic susceptibility to 1541.

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- which indicates that these forms are kinetically similar. The EC_{50} for this transition from procaspase-3 to active caspase-3 based on Michaelis-Menton analysis is 1.5 μ M (fig. S5) and closely matches the EC_{50} of 1.3 μ M based on activity measurements with the preferred substrate Ac-DEVD-AFC (Fig. 1A).
28. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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Materials and Methods
Figs. S1 to S11
References

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High Diversity of the Viral Community from an Antarctic Lake

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Viruses are the most abundant biological entities and can control microbial communities, but their identity in terrestrial and freshwater Antarctic ecosystems is unknown. The genetic structure of an Antarctic lake viral community revealed unexpected genetic richness distributed across the highest number of viral families that have been found to date in aquatic viral metagenomes. In contrast to other known aquatic viromes, which are dominated by bacteriophage sequences, this Antarctic virus assemblage had a large proportion of sequences related to eukaryotic viruses, including phycodnaviruses and single-stranded DNA (ssDNA) viruses not previously identified in aquatic environments. We also observed that the transition from an ice-covered lake in spring to an open-water lake in summer led to a change from a ssDNA- to a double-stranded DNA-virus-dominated assemblage, possibly reflecting a seasonal shift in host organisms.

Antarctica has been geographically isolated for millions of years, harboring some of the last pristine ecosystem on Earth (1–3). Liquid water is represented in Antarctica by temporally or perennially ice-covered lakes and streams (4). Life in Antarctic freshwater ecosystems is dominated by microorganisms that are adapted to extreme environmental conditions: low temperatures, low nutrient levels, and months of nearly complete darkness during winter (1, 5–7).

Viruses are important factors regulating the structure of microbial communities. Virus-mediated killing is implicated in algal bloom control, is considered as important as protozoan grazing in bacterial mortality in oceans, and thus influences

aquatic food-web interactions to affect global geochemical cycles (8, 9). Moreover, microbial genetic diversity is shaped by virus-mediated gene transfer and virus host range (10, 11). Until recently, our knowledge of virus diversity has been limited by the difficulty of culturing their hosts, but now new high-throughput methods for sequencing uncultured viral communities (viromes) have been uncovering their marked genetic richness (11–13).

Byers Peninsula (Livingston Island, Antarctica) contains several freshwater lakes in its inland plateau, whose environmental value led to its designation as an Antarctic Specially Protected Area (14) (fig. S1). These lakes are not substantially influenced by maritime fauna (penguins and seals), although small groups of birds such as skuas or terns sporadically visit them. Oligotrophic Antarctic lacustrine habitats such as these ecosystems receive little nutrient input and are dominated by microorganisms forming truncated food webs (5, 7). However, in spite of the relative simplicity of their ecosystems, these lakes harbor a wide range of bacteria, some algae (including mixotrophs), rotifers and protozoa, and a macrozooplankton community including the copepod *Boeckella poppei* and the fairy shrimp *Branchinecta gainii*. The

benthos is dominated by an aquatic moss (*Drepanocladus longifolius*) and metazoans including the chironomid *Parochlus steinenii* and the oligochaete *Lumbricillus healyae* (14). Aquatic organisms populating these unique habitats experience major seasonal shifts as these lakes remain ice covered for at least 9 months per year with dim light conditions and stable inverse stratification of the water column, whereas in summer the lakes are exposed to intense ultraviolet radiation and poor water stratification owing to ice melt.

Microorganism communities are regulated by the availability of nutrients (bottom-up control), the activity of predators (top-down control), and viral lysis. The role of the top-down control in Antarctic lakes appears to be limited and, thus, the ecological role of viruses in these extreme environments may be greater than in temperate lakes (5, 7). Consistently, Antarctic lakes display the highest rate of visibly phage-infected bacteria reported (15); however, identification of Antarctic viruses is limited to some electron micrographs (16, 17). To profile the virus diversity in Antarctic ecosystems and investigate seasonal variations, water was collected from Lake Limnopolar (Byers Peninsula) before (spring) and after (summer) the ice cover melted (18). Electron microscopy (EM) revealed a large diversity of viral morphologies (Fig. 1A), and quantitative analysis showed that, in contrast to previous studies in aquatic environments, viral particles <30 nm in diameter constituted the most abundant viral morphotype in spring (fig. S2). In summer, we observed a reduction of <30-nm particles and an increase of >50-nm particles, including tailed phages and putative phycodnaviruses with ~150-nm capsids. Consistently, flow cytometry analysis of the summer sample showed a population of viruses with staining properties similar to those of large algal phycodnaviruses (fig. S2).

The main constraint of Antarctic virus studies is the lack of genome sequence data. We sequenced 20 million base pairs of viral DNA purified from Lake Limnopolar. The majority of the 89,347 sequences obtained by pyrosequencing showed no significant similarity to GenBank

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sequences [87.6% on average (fig. S3)] or to metagenomic open reading frame [72% on average (table S1)] data sets. This phenomenon is in common with previously published viromes and reveals our limited knowledge of viral diversity, in spite of the large amount of metagenomic data collected from uncultured microorganisms. Accordingly, <3% of the Antarctic sequences showed any similarity to each of the 30 viromes obtained from aquatic environments by pyrosequencing, with freshwater lake viromes being the most similar (table S2) (12, 13). The sequences ascribed to cellular organisms (table S3) might be due to a poor representation of viruses in databases, contamination with small-size prokaryotes, unidentified prophages annotated as bacterial genomes, or host genes incorporated into viral genomes (10, 12).

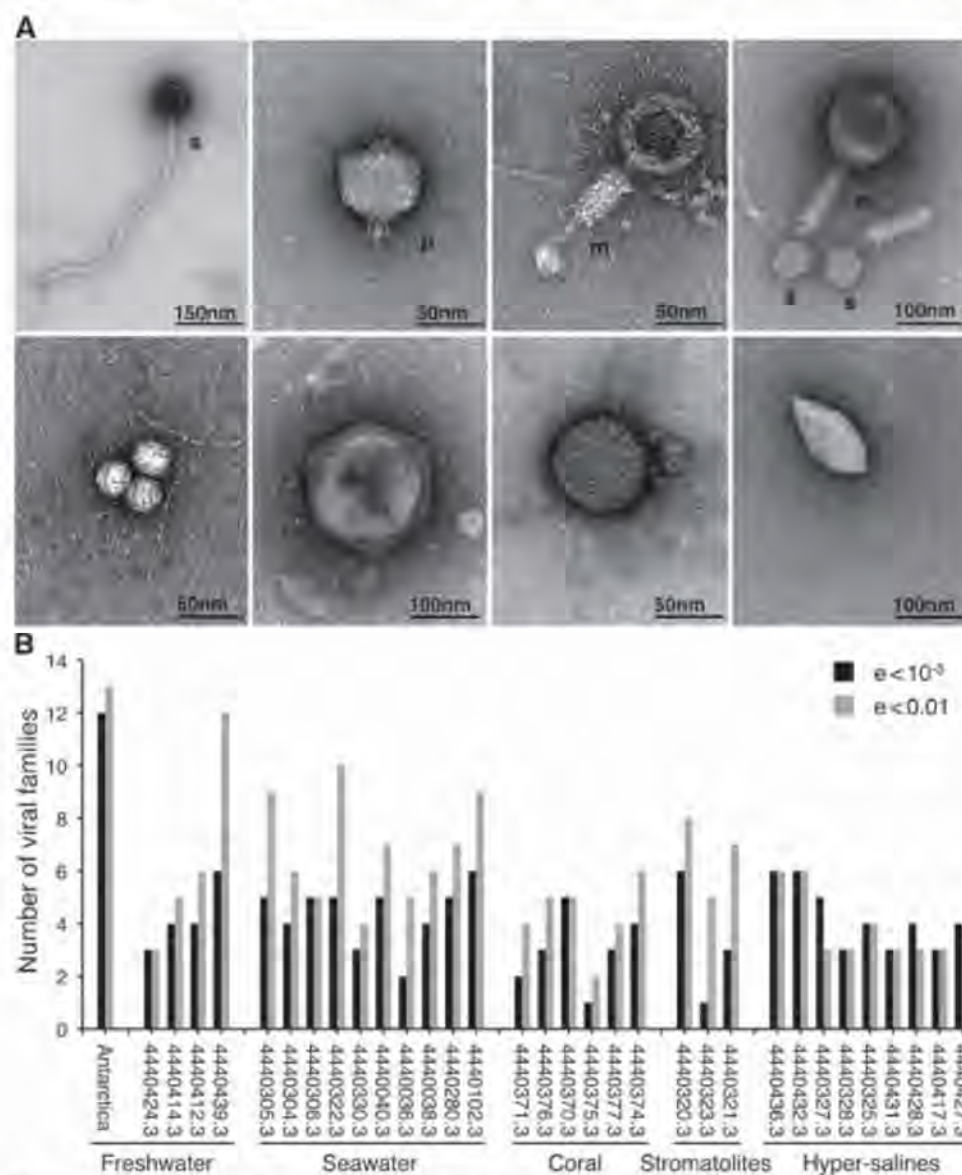
There is evidence that high-latitude ecosystems may have low biological diversity (12, 19, 20).

Lake Limnopolar harbored a marked diversity with viral genotypes distributed across 12 virus families (table S4), greater than that previously found in viromes from aquatic environments, most of them showing the presence of only 3 to 6 viral families (Fig. 1B and table S5). Moreover, prediction of the viral community structure estimated a species richness of 5130 viral genotypes in spring and 9730 in summer, whereas similar estimates of viral genotypes were substantially lower (ranging from 253 to 787) in the few other freshwater viromes available (table S6). This level of diversity of Antarctic viruses was in the high range of that determined for the aquatic, mostly seawater, viromes analyzed so far (12, 21). This unexpected high genetic diversity in Antarctic viruses was also found by polymerase chain reaction (PCR)-amplification within the virus genus T4-myoviruses, one of the most abundant, ubiqui-

tous, and better-studied viruses in environmental samples (table S5) (12, 13, 22). In agreement with the variety of tail phage morphologies seen (Fig. 1A), PCR-amplified sequences of gp23 gene from T4 phages were widely distributed throughout their phylogenetic tree (Fig. 2A and table S7), and most of them had <80% amino acid sequence identity with known sequences.

All of the viromes described to date are dominated by bacteriophages, mostly large-tailed double-stranded (dsDNA) phages (myovirus, podovirus, and siphovirus) and small single-stranded DNA (ssDNA) microvirus (table S4) (12, 21). However, the Antarctic freshwater viral assemblage reported here has a distinct composition with a large proportion of ssDNA viruses, mostly related to eukaryotic viruses. The Lake Limnopolar spring virome was dominated by ssDNA viruses (Fig. 2B), correlating with 56% of viral particles <30 nm observed by EM (fig. S2). Although some Antarctic ssDNA viruses are related to microviruses commonly found in other viromes and phylogenetically related to the chlamydia microvirus genus (Fig. 2B, fig. S4, and table S5) (12, 13), the Antarctic virome contains ssDNA viruses related to other viral families (circovirus, geminivirus, nanovirus, and satellites) that we have not previously seen reported for aquatic environments. Unlike microviruses that infect bacteria, these ssDNA viruses infect mammals, birds, or plants, whose virtual absence in Lake Limnopolar (14) leads us to propose that they infect other eukaryotic hosts. These sequences exclusively aligned to a highly conserved domain (pfam2407) of replication-related genes, and a phylogenetic analysis based on pfam2407 clustered most of these viruses distinctly from the related families of ssDNA viruses and from environmental sequences (Fig. 3A) (23). The abundance of ssDNA virus sequences allowed us to assemble 10 circular ssDNA genomic elements, including the replication protein and stem loop, that are highly conserved among nanoviruses, satellites, and circoviruses (Fig. 3B and table S8). The presence of genomic organizations with two ORFs transcribed unidirectionally, as well as their phylogenetic distance, suggests that these viruses may belong to previously undescribed circular ssDNA viral families. We also assembled 25 other circular DNA genomic elements with no similarity to known sequences (table S8). The host range and ecology of these small DNA viruses is unknown.

In spring under ice cover, the lake virome was dominated primarily by ssDNA viruses and secondarily by dsDNA Caudovirales. The representation of ssDNA viruses decreased after the ice cap thawed in summer, and the viral assemblage became dominated by dsDNA viruses belonging mostly to the Phycodnaviridae family, but also contained Caudovirales and Mimiviridae (Fig. 2B and table S4). Nearly 87% of the phycodnavirus sequences from the summer virome matched a large portion of the genome of OtV5 (prasinovirus genus), a virus infecting the prasinophyceae *Ostreococcus tauri* (24) (fig. S5 and table S9). The presence of an OtV5-



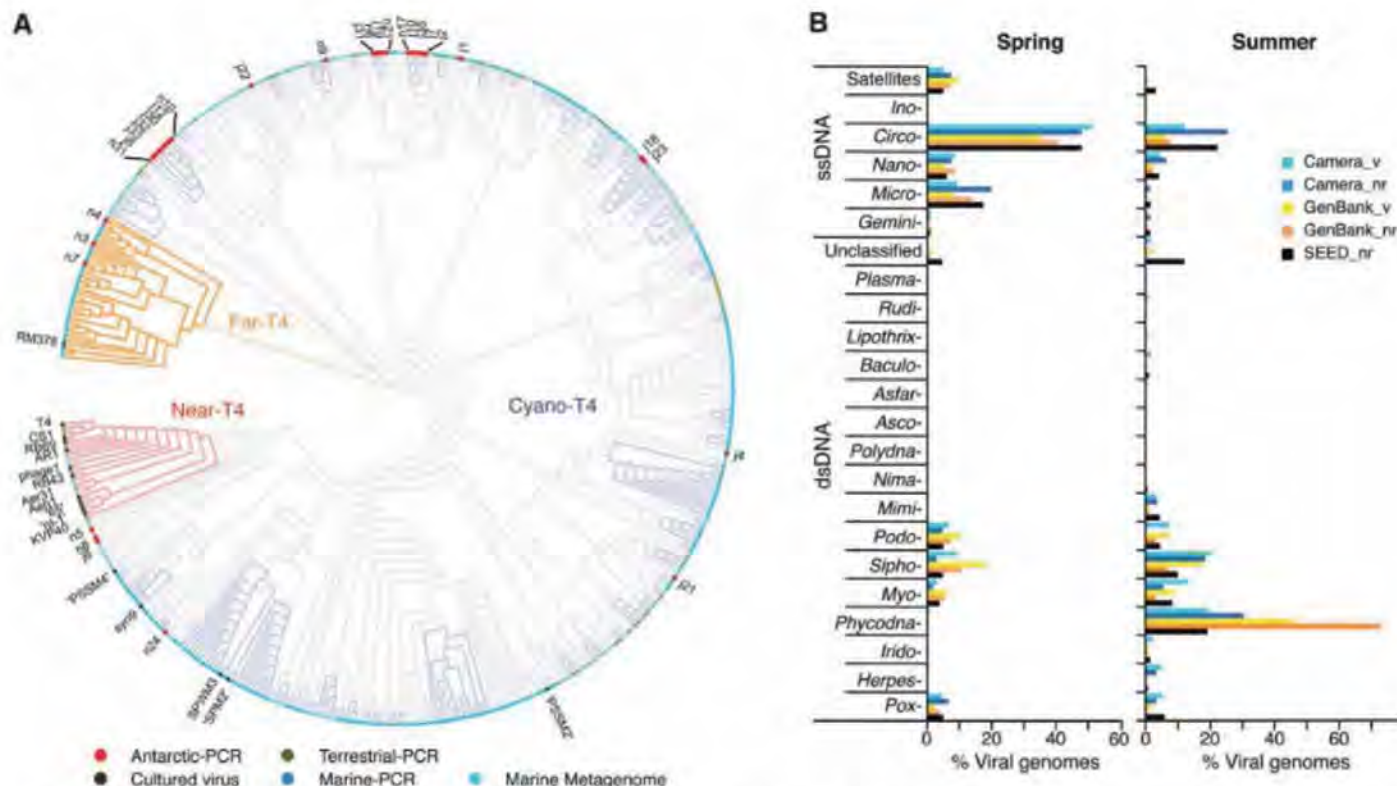
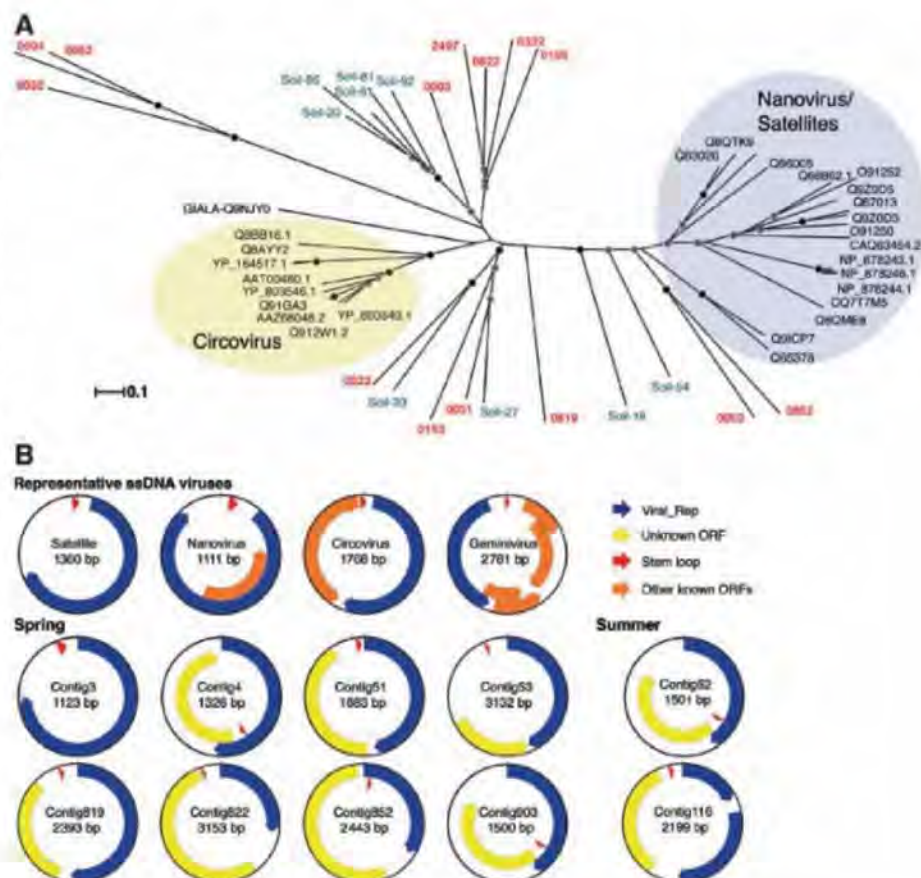


Fig. 2. Genetic diversity of viruses in an Antarctic lake. **(A)** Phylogenetic tree including 30 independent sequences of the gp23 protein of Antarctic T4-myovirus obtained by PCR-cloning in spring and summer. The main groups of T4-phages are indicated by colored lines, and clusters of related sequences supported by internal nodes with >50% bootstrap value are represented by thicker lines. The origin of the sequences is depicted by edge circles colored as

indicated. **(B)** Relative abundance of viral families before (spring) and after (summer) ice melt. The distribution of sequences into viral families, determined by Blast similarity to the indicated database, was corrected by the phi29 polymerase bias toward ssDNA virus genomes [estimated in 100 times (23)] and normalized by genome length to estimate the composition of the viral assemblage.

Fig. 3. Circular ssDNA viruses in Antarctica. **(A)** Phylogenetic tree of replication-related genes with pfam02407, including sequences assembled from Antarctic viromes (red), from rice paddy soil viromes (green) (23), and reference sequences (GenBank number indicated) from ssDNA viruses. Gray and black circles represent internal nodes with >50% and >90% of bootstrap values, respectively. The scale bar indicates the number of amino acid substitutions per residue. **(B)** Genome organization of 10 circular genomic elements assembled from spring and summer Antarctic lake viromes containing a putative replication gene (blue arrow) and a stem loop with a conserved nonanucleotide motif and small reiterated flanked sequences (red arrow). ORFs with no major similarities to known proteins (yellow arrow) and known ORFs not related to replication genes (orange arrow) are indicated. Representative genomes or genomic components of circular ssDNA viruses correspond to satellite (gi 18959285), nanovirus (gi 19744936), circovirus (gi 38018060), and geminivirus (gi 21426900).



related prasinovirus was confirmed by PCR amplification and sequencing of a conserved region encoding its major capsid protein (fig. S4). However, the <78% amino acid identity in the major capsid protein and the fact that some regions of the genome were not identified in spite of the high sequence coverage in the virome indicate that this Antarctic prasinovirus is distinct from OtV5. The change in the viral community composition before and after ice melt, observed with five different search strategies, was consistent with the quantification of virus particles (fig. S2) and probably reflects changes in physical factors and host community (6).

Restricted metabolic activity under the ice promotes lysogeny in large Antarctic phages (15) but may allow the lytic replication of ssDNA viruses with small genomes, which are more abundant in the spring sample. The high prevalence of genes involved in photosynthesis and other cellular metabolic processes in phages and viromes has led others to propose that these genes might facilitate the expansion of their hosts into new ecological niches (25–27). Similarly, the increase we observed in the Antarctic summer virome of genes involved in carbohydrate and amino acid metabolism, respiration, and stress responses suggests that they may help the infected host to better survive the changing environmental conditions after the ice cover melts (fig. S6). Host expansion will also favor virus populations; for example, the phycodnavirus expansion we have observed in the summer sample is probably a consequence of a Prasinophyceae green alga bloom. Scales of the prasinophyte *Pyramimonas geleidicola*, the dominant phytoflagellate in some Antarctic lakes, have been observed as associated with large viral particles (28) in Antarctica. The progressive reduction of Lake Linnopolar ice cover during December provided better radiation transmission to the water column triggering the growth of phytoplankton just under the ice sheet. The algal bloom was detected in December both by a clear chlorophyll *a* peak and a reduction in light adsorption below 1 to 2 m (fig. S7), suggesting that it may represent the early expansion of the algal host of the phycodnavirus that dominated the lake in January (summer sample).

The viral assemblage of Lake Linnopolar reported here is unexpected by comparison with other aquatic environments. First, in contrast with other known aquatic viromes, this Antarctic lake viral community is not dominated by bacteriophages infecting prokaryotes (12, 21) but by viruses known to infect eukaryotes, including ssDNA viruses related to animal and plant viruses, and dsDNA viruses infecting algae. Second, undescribed ssDNA viruses possibly belonging to distinct viral families were found. Third, unprecedented taxonomic diversity and high genetic richness in this Antarctic lake illustrates that high virus diversity may be found in systems where biological diversity in other taxa is low (2, 3, 19). Finally, we found that melting of the ice cover in summer leads to a change in this Antarctic viral community from

small ssDNA to large dsDNA viruses. Altogether, the Lake Linnopolar virome sheds light into the largely unknown community of viruses populating Antarctic freshwater ecosystems.

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29. We thank the Maritime Technology Unit (CSIC) and Las Palmas crew (Spanish Navy) for the logistic help and support that made this expedition possible. We also thank M. Toro, A. Camacho, and other members of the Linnopolar Project for help and discussions; A. Alejo for helpful comments and reviewing the manuscript; and M. Pignatelli for technical support. This work was funded by grants from the Spanish Ministry of Science and Innovation (CGL2005-06549-C02-01/ANT, CGL2007-29843-E/ANT, CTM2008-05134-E/ANT, and BFU2008-04501-E). Spring and summer viral metagenomes from the Antarctic Lake Linnopolar have been submitted to GenBank and assigned the genome project accession number 34669. The metagenomes are also publicly accessible from the ftp server of the SEED public database (<ftp://ftp.theseed.org/metagenomes>) under the project accession numbers 4441778.3 and 4441558.3 for the spring and summer viromes, respectively. PCR-obtained sequences encoding the gp23 protein of Antarctic T4-phages and the MCP protein of Antarctic phycodnavirus have been also deposited at GenBank as "environmental sequences" and are listed under accession numbers FJ791185-247 and FJ791175-84, respectively.

Supporting Online Material

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Materials and Methods
Figs. S1 to S7
Tables S1 to S9
References

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Viral Glycosphingolipids Induce Lytic Infection and Cell Death in Marine Phytoplankton

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Marine viruses that infect phytoplankton are recognized as a major ecological and evolutionary driving force, shaping community structure and nutrient cycling in the marine environment. Little is known about the signal transduction pathways mediating viral infection. We show that viral glycosphingolipids regulate infection of *Emiliania huxleyi*, a cosmopolitan coccolithophore that plays a major role in the global carbon cycle. These sphingolipids derive from an unprecedented cluster of biosynthetic genes in *Coccolithovirus* genomes, are synthesized de novo during lytic infection, and are enriched in virion membranes. Purified glycosphingolipids induced biochemical hallmarks of programmed cell death in an uninfected host. These lipids were detected in coccolithophore populations in the North Atlantic, which highlights their potential as biomarkers for viral infection in the oceans.

Marine phytoplankton are the basis of marine food webs and are responsible for nearly half the global carbon-based net primary production (1). The coccolithophorid *Emiliania huxleyi* (Prymnesiophyceae, Haptophyte) is a cosmopolitan unicellular photoautotroph whose intricate calcite skeletons account for about a third of the total marine CaCO₃ production. *E. huxleyi* forms massive annual blooms in the North Atlantic that have been shown to be infected

and terminated by lytic, giant double-stranded DNA containing coccolithoviruses (2, 3), a subset of the larger *Phycodnaviridae* group that infects microalgae (4). As the most abundant biological entities in aquatic environments, viruses turn over more than a quarter of the photosynthetically fixed carbon, thereby fueling microbial food webs and short-circuiting carbon export to higher trophic levels and the deep sea (5, 6). In addition, marine viruses stimulate the lateral trans-

fer of genes from one host cell to another, which contributes to the diversification and adaptation of plankton in the oceans (7).

Very little is known about the molecular mechanisms and signal transduction pathways mediating phytoplankton cell death by marine viruses. However, it was recently shown that lytic viral infection of *E. huxleyi* induces hallmarks of autocatalytic, programmed cell death (PCD), including metacaspase expression and caspase activity, which were required for successful viral replication (8). The recent availability of genomic resources for an *E. huxleyi* host (9) and *E. huxleyi* lytic virus strain 86 (EhV86) (10) provides an unprecedented opportunity to explore cellular pathways triggered during execution of viral infection and to gain insights into the origin of PCD in these unicellular photoautotrophs. The genome sequence of EhV86, the type strain for the *Coccolithoviruses*, revealed an unexpected cluster of putative sphingolipid biosynthetic genes (10), a pathway that has not been described in a viral genome. Recent phylogenetic evidence for the transfer of seven genes in the sphingolipid biosynthesis pathway between *E. huxleyi* and EhV86 suggests a critical role in host-virus interactions (11). De novo sphingolipid biosynthesis is initiated by serine palmitoyltransferase (SPT) (12) and leads to ceramide production, a potent inducer of PCD in animals and plants (13, 14). The EhV86-encoded SPT gene is expressed during infection (10, 15) and encodes an active SPT with a unique biochemical preference for myristoyl-coenzyme A (myristoyl-CoA) as a substrate, when expressed heterologously in yeast (16). Nonetheless, it is not known whether these lipids play any functional role during infection of *E. huxleyi*.

We examined the polar membrane composition of uninfected and EhV86-infected sensitive (*Ehux374*) and resistant (*Ehux373*) *E. huxleyi* strains during the course of lytic infection. Using high-performance liquid chromatography with electrospray ionization mass spectrometry (HPLC/ESI-MS) (17, 18), we compared the lipid composition of uninfected and infected host cells. We detected glycosphingolipids (GSLs) in uninfected host cells that appeared to be composed of predominantly hydroxyl-sphingoid bases derived from palmitoyl-CoA (Fig. 1A). These host sphingoid bases are consistent with the expected products of the host SPT, which utilizes palmitoyl-CoA, and are common in plants (19). However, the lipids from EhV86-infected *Ehux374* had unique GSLs yielding fragmentation ions that were indicative of multiply hydroxylated sphingoid bases derived from myristoyl-CoA (Fig. 1B and fig. S1). These

sphingoid bases are the expected products of the viral SPT, based on its preference for myristoyl-CoA (16). The virus-induced myristoyl-base GSLs were absent in uninfected cells and were unique to lytic viral infection (Fig. 1B). Both resistant and susceptible hosts produced significant concentrations of host palmitoyl GSLs, which were structurally distinct from the viral myristoyl GSLs (Fig. 1, A and D).

To ascertain the origin of the myristoyl GSLs, viruses were purified using a cesium chloride (CsCl_2) density gradient and ultracentrifugation. Identical myristoyl GSLs were dominant components of the lipids extracted from purified EhV86 viruses (Fig. 1C); host palmitoyl GSLs were absent. These observations indicate that the viral GSLs are a component of the membranes packaged

with this virus. Although GSLs are distributed in some prokaryotes, they have not been found in phytoplankton or microalgal viral membranes (20). Proteomic analysis of the EhV86 virion determined that 23 out of 28 proteins are predicted to be membrane proteins (21), which corroborates our observations of GSLs and membrane structures. Sphingolipids, such as GSLs, are common constituents of membrane lipids, which may form lipid rafts in eukaryotes. For example, lipid rafts may be involved in the entry and budding of HIV and hepatitis C, yet their role is not well understood (20, 22). Transmission electron microscopy (TEM) micrographs of infected *Ehux374* cells revealed viral particles within intracytoplasmic vacuoles, consistent with this type of strategy (Fig. 1B, inset).

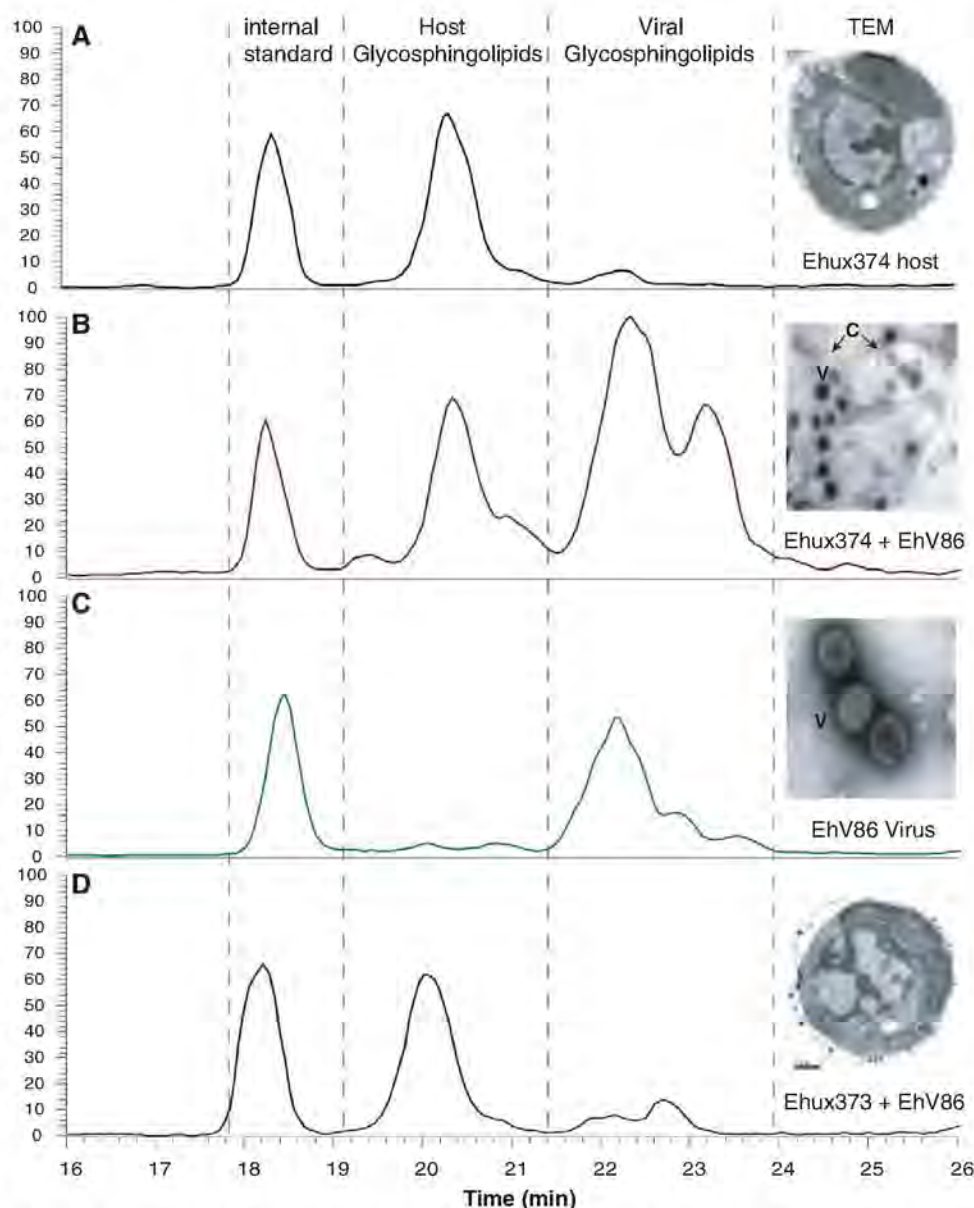


Fig. 1. Production of viral GSLs in EhV86-infected *E. huxleyi* cells and in purified EhV86 virions. Summed ion HPLC/MS chromatograms showing relative abundances (normalized to an internal standard) of GSLs extracted from (A) susceptible *Ehux374*, (B) *Ehux374* infected with EhV86 52 hours post infection, (C) purified EhV86 on a CsCl_2 gradient, and (D) resistant *Ehux373* infected with EhV86 52 hours post infection. (Insets) TEM micrographs of respective treatments. Arrows in (B) depict intracytoplasmic vacuoles (V) that contain viral particles (V).

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The accumulation of viral GSLs in *Ehux374* during EhV86 infection was accompanied by a reduction in cell abundance, a severely compromised photochemical quantum yield of photosystem II (PSII) (declining to 0.22 after 48 hours), and an ~30-fold induction in caspase specific activity (Fig. 2). Induction of this biochemical PCD marker occurred concomitantly with de novo synthesis of viral GSLs and viral replication (reaching 3×10^8 virus/ml), leading to the demise of *Ehux374* at the onset of the lytic phase 25 hours post infection. Although elevated viral GSLs were detected at 3.5 hours in EhV86-infected cultures, presumably because of the presence of free viruses, de novo production of viral GSLs began after the first 12 hours (Fig. 2D, inset), trailing gene expression dynamics of the virally encoded SPT at 2 hours post infection (15). In late infection (>50 hours), caspase specific activity and GSL production exceeded levels seen in uninfected cells or in resistant *Ehux373* cells by a factor of >100 (Fig. 2, C and D). Linear regression demonstrated a high correlation ($R^2 = 0.815$) between viral GSL production and caspase specific activity over the course of lytic viral infection (fig. S2). In contrast, the EhV86-resistant strain *Ehux373* exhibited slightly better growth than control, uninfected *Ehux374* cells (Fig. 2A). Only trace levels of viral GSLs were detected in infected *Ehux373*, probably originating from the viral inoculum (Figs. 1D and 2D).

We monitored the ability of viral GSLs to modulate host physiology after purification from EhV86-infected *Ehux374* cells by preparative HPLC and addition to uninfected *Ehux374* at various concentrations (Fig. 3). The viral GSLs suppressed cell growth compared with control cells treated with dimethyl sulfoxide (DMSO, a solvent) and control cells treated with phosphatidylglycerol (PG), which had a similar HPLC retention time

(Fig. 3A). The cells treated with viral GSLs exhibited a dose-dependent induction of cell death above a threshold concentration ($>0.06 \mu\text{g/ml}$). Induction of cell death compromised photosynthetic efficiency of PSII (Fig. 3A) and resulted in elevated in vivo caspase activity for about 20 to 25% of *Ehux374* cells treated with 0.3 and 1.5 $\mu\text{g/ml}$ of viral GSLs after 48 hours (Fig. 3B). The induction of in vivo caspase activity was assessed by cell staining with the fluorescently labeled caspase probe, VAD-FMK conjugated with fluorescein isothiocyanate (FITC), and flow cytometric analysis (Fig. 3B) (18). Likewise, 18 and 56.2% of the cells at these two concentrations were positively stained with SYTOX, a DNA binding fluorescent indicator of compromised cell membrane integrity (Fig. 3C); this observation is consistent with previous findings of late stages of PCD in phytoplankton (23). Only 5.6 to 10.3% of cells were positively stained with VAD-FMK-FITC in the control treatments and in cells that were treated with a sublethal GSL concentration (e.g., 0.06 $\mu\text{g/ml}$), which indicated minimal induction of PCD. Likewise, a visual comparison of control (DMSO or PG) and GSL treatments revealed massive cell lysis only at GSL concentrations $>0.06 \mu\text{g/ml}$ (Fig. 3C).

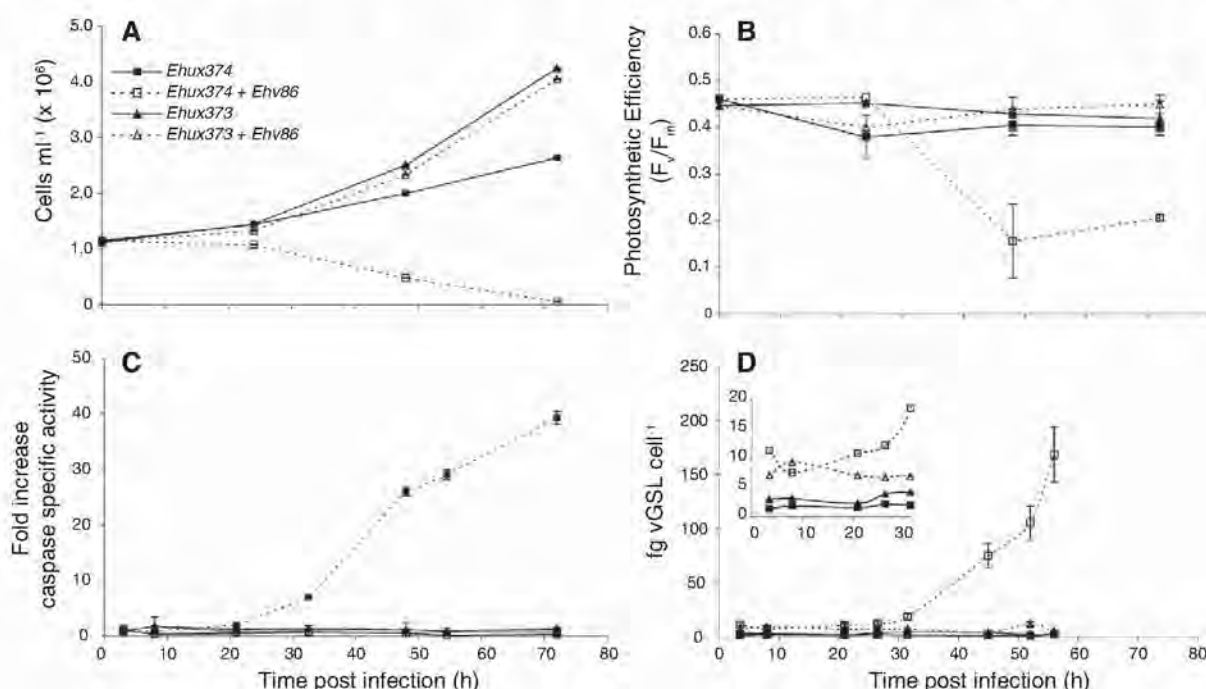
Given their potent ability to trigger *E. huxleyi*'s PCD response in a dose-dependent manner and their presence in purified EhV virions, we propose that viral GSLs may be part of a timing mechanism for viral release. In such a mechanism, host lysis is dependent on the accumulation of viral myristoyl GSLs to a critical effective concentration, above which host PCD is induced. According to our measurements, an EhV86 virion contains ~0.1 to 0.3 fg of myristoyl GSLs. At a typical burst size of ~800 to 1000 viruses per cell (8), an effective intracellular concentration of about 80 to 300 fg

per cell is reached at the peak of the lytic phase; this concentration is consistent with the 100 to 200 fg per cell observed at the onset of lytic infection and PCD activation (>50 hours post infection) (Fig. 2, C and D, and fig. S3). Given that ceramide enrichment in cell membranes can serve to modulate entry and release of viruses (20, 22), GSL enrichment in intact EhV86 virions and the profound accumulation of viral GSL during lytic phase may suggest a similar mechanism for the timed release of EhV86 virions.

We hypothesize that such bioactive molecules have the potential also to elicit cell death in surrounding, uninfected cells under natural bloom densities and, hence, may serve as a bloom termination signal. It has been suggested that induction of PCD in *E. huxleyi* can act as a "viral exclusion" strategy (8, 24) to limit production of viruses during infection and, ultimately, their propagation through clonal populations. We calculated the "sphere of influence" distance around a given lysed cell that would contain an adequate viral GSL concentration to induce PCD in surrounding cells. Using our measurements of viral GSL concentrations (~200 fg per cell) and a threshold concentration of 0.2 $\mu\text{g/ml}$ to induce PCD and cell lysis (fig. S4) and assuming the GSL is isotropically dispersed, we estimated that a sphere with a diameter of a few hundred micrometers would be sufficient to elicit cell death. Given that *E. huxleyi* blooms can reach about 100,000 cells/ml (25) and that cells clearly respond to external GSL application (Fig. 3), it is plausible that GSLs act as intercellular signals. Similar findings were recently reported for diatom-derived oxylipins found to act as infochemicals either to potentiate PCD or to induce resistance in sublethal doses (26, 27).

Because of their potent bioactivity and unique chemical signature, we also propose virus-derived

Fig. 2. Onset of the lytic phase during EhV86 infection is mediated by induction of caspase activity and viral GSL production. Viral infection dynamics of susceptible *Ehux374* or resistant *Ehux373* strains as monitored by the following parameters: (A) host abundance; (B) photochemical quantum yield (F_v/F_m) of PS II, a proxy for photosynthetic health; (C) caspase specific activity via normalized cleavage of IETD-AFC in cell extracts; and (D) de novo synthesis of viral, myristoyl GSLs. Data in (A) to (C) is derived from four to six biological replicates for infected cultures. Analytical error for GSL measurement is from two independent experiments, each analyzed in triplicate.



GSLs can act as a novel biomarker for viral infection of natural phytoplankton assemblages. We collected natural plankton for GSL analyses during a cruise in the North Atlantic along 65°W between 43°N and 33°N during April of 2008. We used concentrations of 19'-hexanoxyfucoxanthin (19'-hex), a pigment marker for prymnesiophytes, including coccolithophores, as a proxy for host abundances (Fig. 4). Palmitoyl GSLs, similar to those seen in uninfected *Ehux374* cultures, were observed throughout the North Atlantic transect at stations characterized by high 19'-hex pigment

concentrations, which we postulate are indicative of healthy coccolithophore populations. In contrast, at a local minimum in 19'-hex pigment concentrations, we observed a GSL molecule with an HPLC retention time and mass spectrum that was consistent with the myristoyl GSL observed in EhV86-infected cultures of *Ehux374* (compare Fig. 1, Fig. 4, and fig. S3). Together with our laboratory-based findings showing viral GSL production during viral lysis and its incorporation into virions (Fig. 1, 2), the observation of a putative viral GSL at the 19'-hex minimum is

suggestive of virus-induced demise of a natural *E. huxleyi* population at this location. Detected expression of some host and viral sphingolipid biosynthetic genes in natural *E. huxleyi* populations corroborate our findings (28). On the basis of the diagnostic preference of the viral SPT for myristoyl-CoA (16), these viral GSLs may serve as a proxy for viral infection of coccolithophore populations in the sea. Currently, biomarkers to quantify active viral infection in the oceans are lacking, which hinders our understanding of the role and activity of viruses and virus-mediated

Fig. 3. Application of purified viral GSL to uninfected *E. huxleyi* cells mimics infection by inducing PCD. Dose-dependent induction of cell death in uninfected *Ehux374* cells over 72 hours by application of purified GSLs. **(A)** Cell abundance (bars) and photochemical quantum yield of PS II (circles). Error bars, standard deviation of four biological replicates. **(B)** In vivo caspase activity (measured by flow cytometry). Cytograph plots represent the fluorescence distribution of 5000 cells 48 hours post treatment and after staining with VAD-FMK-FITC (CaspACE). The percentage of positively stained cells is given. The dashed line represents the threshold fluorescence above which cells are positively stained (as determined by unstained cells for each treatment). **(C)** Viral GSL-treated cultures exhibited massive cell lysis after 72 hours. The percentage of SYTOX-positive cells, which serves as a proxy for dying cells, is given. Control treatments consisted of DMSO (solvent) or phosphatidylglycerol (PG).

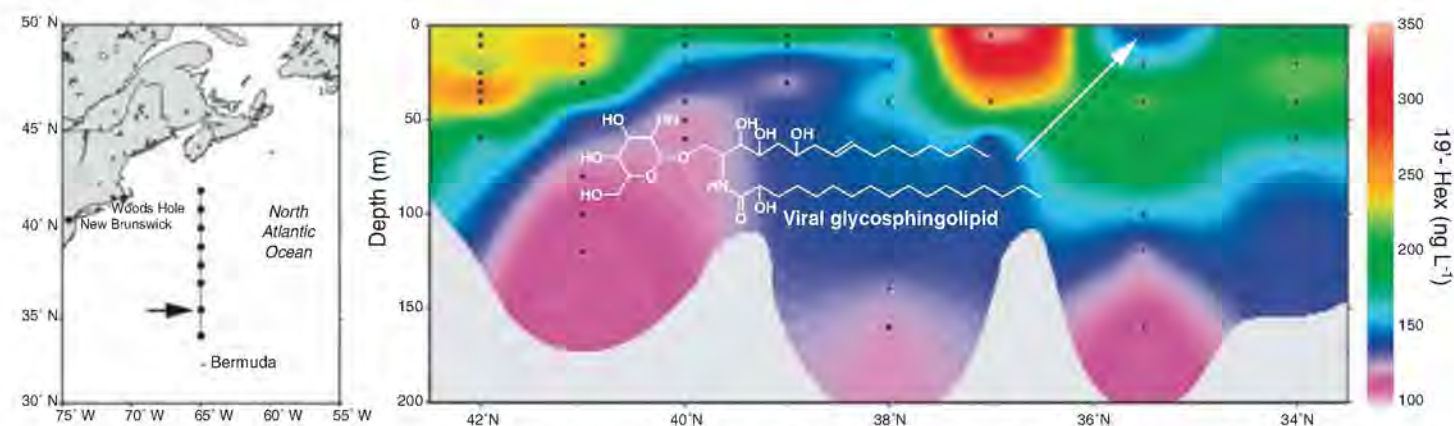
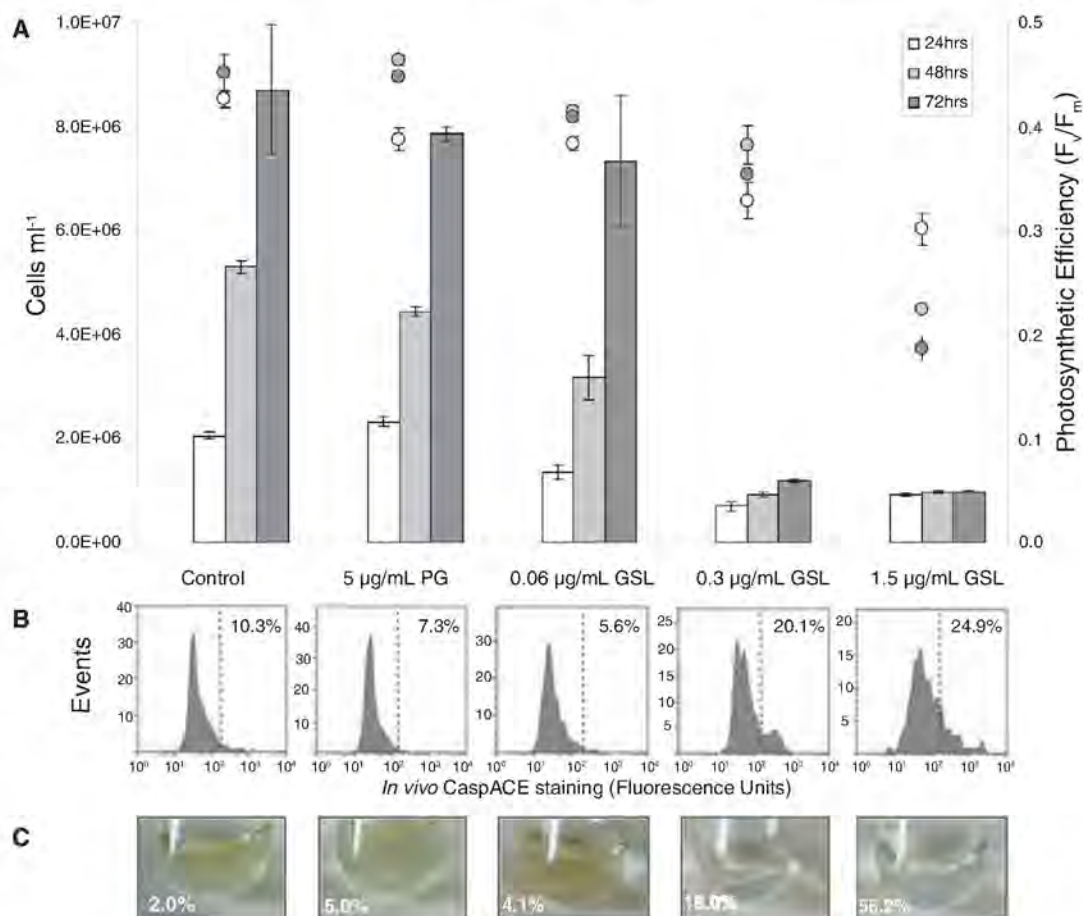


Fig. 4. Viral GSLs represent a potential biomarker for viral infection of natural coccolithophore populations. Vertical distribution of 19'-hex along a meridional transect at 65°W in the North Atlantic (43°N to 33°N). A multiply hydroxylated myristoyl GSL, which we posit is of viral origin, was

only detected in a distinct zone of minimum 19'-hex in pigment values (35°N 65°W, 5-m depth; designated by arrows). This observation is consistent with population demise by viral infection. **(Inset)** Tentative chemical structure of the observed myristoyl GSL.

processes in the oceans. We posit that future studies will elucidate the relative importance of biotic (viral infection as in this study) and abiotic [phosphorus limitation (17)] stress conditions, in shaping community structure of marine microbes, through the detection of different classes of stress-specific lipids.

Although the origin of PCD in unicellular organisms is still unclear, its functional conservation among phylogenetically diverse phytoplankton lineages suggests key evolutionary and ecological drivers in aquatic environments (29). The retention and expression of a nearly complete, virus-based sphingolipid biosynthetic pathway, along with its requirement for viral replication and regulation of the host cell fate, now underscores the pivotal role of a chemical-based, coevolutionary "arms race" in mediating host-virus interactions.

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Supporting Online Material

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Materials and Methods

SOM Text

Figs. S1 to S4

References

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Genome Sequence, Comparative Analysis, and Population Genetics of the Domestic Horse

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We report a high-quality draft sequence of the genome of the horse (*Equus caballus*). The genome is relatively repetitive but has little segmental duplication. Chromosomes appear to have undergone few historical rearrangements: 53% of equine chromosomes show conserved synteny to a single human chromosome. Equine chromosome 11 is shown to have an evolutionary new centromere devoid of centromeric satellite DNA, suggesting that centromeric function may arise before satellite repeat accumulation. Linkage disequilibrium, showing the influences of early domestication of large herds of female horses, is intermediate in length between dog and human, and there is long-range haplotype sharing among breeds.

As one of the earliest domesticated species, the horse, *Equus caballus*, has played an important role in human exploration of novel territories. Belonging to the order perissodactyla (i.e., odd-toed animals with hooves), the genus *Equus* radiated into 8 or 9 species around three million years ago (1). Members of the family equidae exhibit diverged karyotypes (2) and variable centromeric positioning (1). With over 90 hereditary conditions, which may serve as models for human disorders (3, 4) (such as infertility,

inflammatory diseases, and muscle disorders), the horse has much to offer as a model species.

DNA from a single mare of the Thoroughbred breed was sequenced to 6.8× coverage [supporting online material (SOM) text], resulting in a high-quality draft assembly (designated EquCab2.0) with a 112-kb N50 contig size (SOM) and a 46-Mb N50 scaffold size (tables S1 and S2), and >95% of the sequence anchored to the 64 (2N) equine chromosomes. The 2.5- to 2.7-Gb genome size is somewhat larger than the dog genome (2.5 Gb)

and smaller than the human and bovine genomes (2.9 Gb) (5–7). Segmental duplications (8) make up <1% of the equine genome, and most are intrachromosomal duplications such as are seen in many other mammalian genomes (SOM). Repetitive sequences, many equine-specific, make up 46% of the genome assembly (SOM). The predominant repeat classes include long interspersed nuclear elements, dominated by L1 and L2 types (tables S3 and S4) (19% of bases), and short interspersed nuclear elements, including the recent ERE1 and ERE2 and the ancestral main immunogenic regions (7% of bases). Comparison of horse and human chromosomes reveals strong conserved synteny between these species (fig. S1). Indeed, 17 horse chromosomes (53%) comprise material from a single human chromosome (in the dog, it is 29%).

One unexpected feature of the horse genome landscape was the identification of an evolutionary new centromere (ENC) on chromosome 11 (ECA11), captured in an immature state. Several ENCs have been generated in the genus *Equus* by centromere repositioning (a shift of centromeric position without chromosome rearrangement) (1). Mammalian centromeres are typically complex structures characterized by the presence of satellite tandem repeats. ENCs are believed to form initially by unknown mechanisms in repeat-free regions and then progressively acquire extended arrays of satellite tandem repeats that may contribute to functional stability (9). The centromere of ECA11 resides in a large region of conserved synteny in many mammals, where the horse is the only species with a centromere present, strongly suggesting that this centromere is evolutionarily new. The ECA11 centromere is the only horse centromere lacking any hybridization signal in fluorescence in situ hybridization

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experiments probing with the two major horse satellite sequences (fig. S2A, table S8, and SOM text), as if it had not had enough time to acquire satellite DNA. We cytogenetically localized the primary constriction (fig. S2B), then precisely mapped, at the sequence level, the centromeric function using chromatin immunoprecipitation (ChIP)-on-chip experiments (fig. S5). In this region, we found only five sequence gaps [none >200 base pairs (bp)], no protein coding sequences, normal levels of noncoding conserved elements, and typical levels of interspersed repetitive sequences, but no satellite tandem repeated sequences (Fig. 1A). We also found no evidence of accumulation of L1 transposons (10) or KERV-1 elements (11), which were previously hypothesized to influence ENC formation. We propose that the ECA11 centromere was formed very recently during the evolution of the horse lineage, and, in spite of being functional and stable in all horses, has not yet acquired the marks typical of mammalian centromeres.

The equine gene set is similar to those of other eutherian mammals and has a predicted 20,322

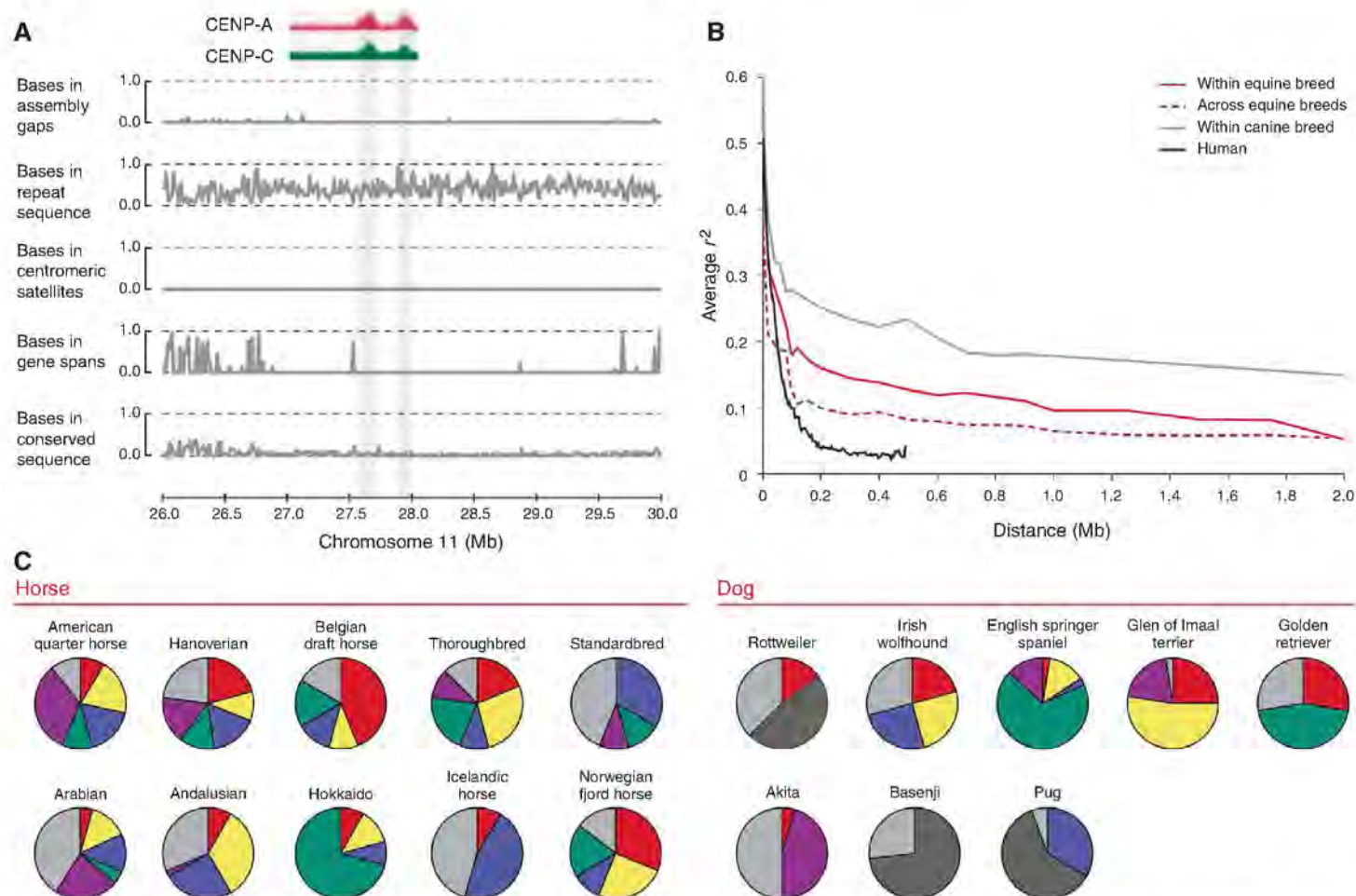


Fig. 1. Major findings of the genome analysis. **(A)** Analysis of the primary centromeric constriction of ECA11: 26,000,000 to 30,000,000 bases. ChIP-on-chip analysis with antibodies against centromeric proteins (CENP-A and CENP-C) shows two regions (136 and 99 kb) bound by kinetochore proteins. There are no uncaptured and few captured gaps, a normal fraction of bases in repeat sequences, no satellite tandem repeats, no protein-coding sequences present nearby, and normal levels of noncoding conserved elements (29

eutherians). **(B)** Horse LD is intermediate between human and dog. **(C)** Horses exhibit more long-range across-breed haplotype sharing than dogs. Haplotypes have the same color across breeds. Haplotypes in <5% of all individuals are light gray, and haplotypes in >5% of all individuals but a single breed are dark gray. Data show LD regions on ECA18 (first 100 kb) and dog chromosome 12 (first 100 kb), which are representative. Full data are in table S11.

protein-coding genes (ENSEMBL build 52.2b), of which 16,617, 17,106, and 17,106 have evidenced orthology to human, mouse, and dog, respectively. The remainder is composed of projected protein-coding genes, novel protein-coding genes, and pseudogenes. One-to-one orthologs with the human account for 15,027 horse gene predictions (SOM). Transcriptome analysis of eight equine samples confirmed the expression of 87% of the 18,039 nonoverlapping genes predicted by ENSEMBL and 88% of the 169,073 predicted exons. Gene family analysis shows paralogous expansion in horses as compared to both human and bovine (SOM) for several interesting families, such as keratin genes related to the condition of pachyonychia (nail bed thickening) in humans (12), perhaps affecting hoof formation; and opsin genes for photoreception, possibly advantageous for visual perception of predators (table S9).

The history of horse domestication, which has important implications for trait mapping strategies, differs in important ways from that of the domestic dog but is perhaps similar to that of the cow. Horses do not appear to have undergone a tight domestication bottleneck, and the presence of many matrilineal lines in domestic horse history has been postulated (13). Screening the horse Y chromosome revealed a limited number of patrilineal lines, consistent with a strong sex bias in the domestication process (14).

We first generated a single-nucleotide polymorphism (SNP) map of more than one million markers at an average density of one SNP per 2 kb by lightly sequencing seven horses from different breeds and by mining the assembly for SNPs (table S10).

We characterized the haplotype structure within and across breeds by genotyping 1,007 SNPs from 10 regions of the genome (SOM) in 12 populations, including 11 breed sets (each with 24 representatives), and 1 set of individual representatives from 24 other breeds and equids. 98% of SNPs were validated, with an average of 69% being polymorphic in alternate breeds (SOM). Like the bovine (15), within-breed linkage disequilibrium (LD) is moderate, dropping to twice the background levels (r^2) at 100 to 150 kb (Fig. 1B). The majority of breeds showed similar LD (SOM and fig. S7), and major haplotypes were frequently shared among diverse populations (Fig. 1C). Based on the length of LD in the horse, the number of haplotypes within haplotype blocks, and the polymorphism rate, power calculations suggest that ~100,000 SNPs are sufficient for association mapping within all breeds as well as across breeds (SOM and fig. S8).

Phylogenetic relationships among breeds were inconsistent across resequenced regions (fig. S9), which is most likely a consequence of the close relationships of horse breeds worldwide. We were unable to phylogenetically separate *E. przewalskii* from the domesticated horses, despite its different karyotype (2N = 66 versus 2N = 64 for the domesticated horse), which is in agreement with recent findings (16), whereas the donkey (*E. africanus*) is clearly a distinct taxon (fig. S9, table S14, and SOM text). This suggests that

either intermixing of *E. przewalskii* and *E. caballus* occurred after subspecies separation or that *E. przewalskii* is recently derived from *E. caballus*.

We demonstrated the utility of the equine genome sequence and a SNP map by applying these resources to mutation detection for the Leopard Complex (LP) spotting locus (SOM). LP (Appaloosa spotting) is defined by patterns of white occurring with or without pigmented spots (fig. S10). Homozygosity confers a phenotype associated with congenital stationary night blindness in the Appaloosa breed (17). Fine mapping of a 2-Mb region followed by regional sequence capture and sequencing (300 kb) found no indications of associated copy number variants or insertions or deletions but found 42 associated SNPs. Of these, 21 reside within an associated haplotype near a candidate gene *melastatin 1* (*TRPM1*), which is expressed in the eye and melanocytes (18). Two conserved SNPs may be good candidates for the causal mutation.

Our analysis of the first high-quality draft sequence of a horse (*E. caballus*) distinguishes *E. caballus* from earlier eutherian genomes by its large synteny with humans and the identification of a centromere repositioning event that may provide an effective model to study epigenetic factors responsible for centromere function. Our results demonstrate that horse population history has led to across-breed haplotype sharing, increasing the feasibility of across-breed mapping. Mapping projects in the horse are likely to accelerate in the coming years and will identify mutations in genes related to morphology, immunology, and metabolism, which may benefit human health.

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Supporting Online Material

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Materials and Methods

SOM Text

Figs. S1 to S11

Tables S1 to S14

References

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MafB/c-Maf Deficiency Enables Self-Renewal of Differentiated Functional Macrophages

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In metazoan organisms, terminal differentiation is generally tightly linked to cell cycle exit, whereas the undifferentiated state of pluripotent stem cells is associated with unlimited self-renewal. Here, we report that combined deficiency for the transcription factors MafB and c-Maf enables extended expansion of mature monocytes and macrophages in culture without loss of differentiated phenotype and function. Upon transplantation, the expanded cells are nontumorigenic and contribute to functional macrophage populations in vivo. Small hairpin RNA inactivation shows that continuous proliferation of MafB/c-Maf deficient macrophages requires concomitant up-regulation of two pluripotent stem cell-inducing factors, KLF4 and c-Myc. Our results indicate that MafB/c-Maf deficiency renders self-renewal compatible with terminal differentiation. It thus appears possible to amplify functional differentiated cells without malignant transformation or stem cell intermediates.

The nonproliferative state of terminally differentiated cells is assured by robust, often redundant mechanisms (1, 2), and in rare exceptions where fully mature cells can re-enter the cycle, proliferation remains transient and/or

involves de-differentiation (3). It remains unknown what renders differentiated cells refractory to the same mitogen signals that stimulate the proliferation of their direct precursors. For example, the proliferative response of myelomonocytic

progenitors to macrophage colony-stimulating factor (M-CSF) is lost upon differentiation to macrophages (4), despite the continued ability of these mature cells to sense the cytokine (5). Consequently, myeloid progenitor cells form colonies in M-CSF containing semisolid medium, whereas blood monocytes and tissue macrophages do not. Here, we have investigated whether this process involves the transcription factors MafB and c-Maf, which can both regulate M-CSF responsiveness (6, 7) and stimulate monocytic differentiation (8–10).

Intriguingly, we observed that in contrast to wild-type (WT) cells, MafB/c-Maf double defi-

cient (Maf-DKO) blood leukocytes formed colonies in M-CSF containing medium at high efficiency (Fig. 1A). Several lines of evidence indicated that these colonies were initiated by mature monocytes rather than by other mature cell types or circulating progenitors. First, in a cytokine mix that can reveal rare circulating stem and progenitor cells (11), Maf-DKO leukocytes gave rise to the same low number of colonies as WT cells (fig. S1A). Furthermore, M-CSF colonies did not develop from lymphocytes or granulocytes (fig. S1C) but formed at very high frequency from purified mature Maf-DKO monocytes that expressed Mac-1, F4/80, and CD115 but were negative for c-kit (CD117), a marker of both primitive (11) and M-CSF-responsive macrophage/dendritic cell progenitors (12) (Fig. 1B and fig. S2). A high rate of colony formation was also observed for spleen and peritoneal macrophages (fig. S1B) as well as for purified CD117⁺ Kupffer cells of the liver, which represent terminally differentiated tissue macrophages (Fig. 1B). These

results indicated that, in contrast to WT, mature Maf-DKO blood monocytes and tissue macrophages could proliferate in response to M-CSF.

Indeed, when we injected recombinant M-CSF directly into the circulation of mice, we observed that Maf-DKO, but not WT, blood monocytes had entered the cell cycle (Fig. 1C). The same observation was made when cells were stimulated with M-CSF *ex vivo* (fig. S1D). Although Maf-DKO monocytes or *in vitro* differentiated macrophages required significantly higher M-CSF concentrations for proliferation than bone marrow progenitors (fig. S3), their ability to divide was not restricted to a small number of cycles but continued in extended long-term culture. Maf-DKO monocyte-derived colonies could thus be serially replated in methocult assays at high efficiency and without loss of clonogenicity (Fig. 1D). This was intriguing, because even progenitors normally have only limited replating ability in this assay that is often employed to reveal the extended self-renewal capacity of transformed progenitors (13). Moreover, extended,

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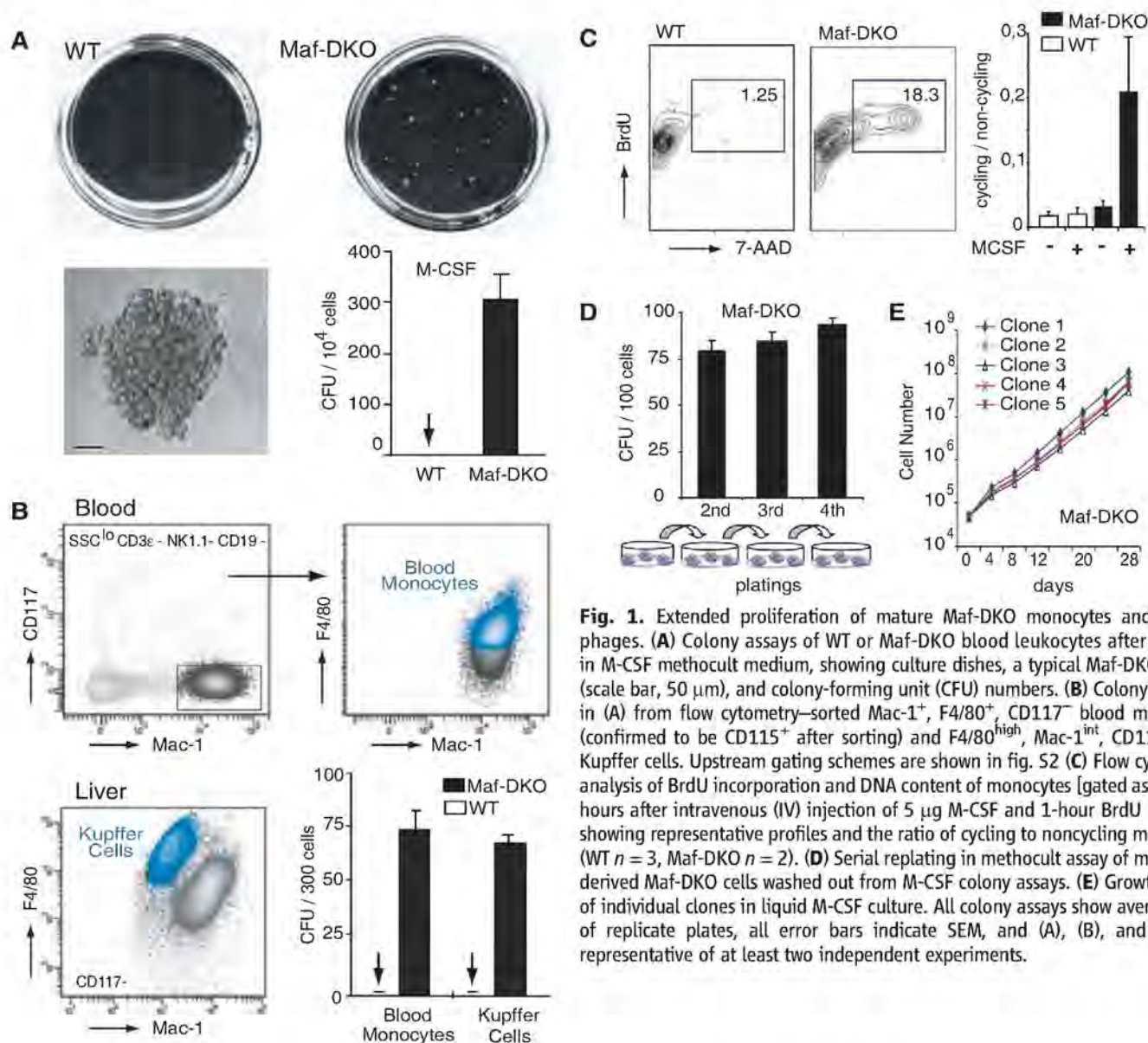


Fig. 1. Extended proliferation of mature Maf-DKO monocytes and macrophages. **(A)** Colony assays of WT or Maf-DKO blood leukocytes after 12 days in M-CSF methocult medium, showing culture dishes, a typical Maf-DKO colony (scale bar, 50 μ m), and colony-forming unit (CFU) numbers. **(B)** Colony assay as in **(A)** from flow cytometry-sorted Mac-1⁺, F4/80⁺, CD117[−] blood monocytes (confirmed to be CD115⁺ after sorting) and F4/80^{high}, Mac-1^{int}, CD117[−] liver Kupffer cells. Upstream gating schemes are shown in fig. S2. **(C)** Flow cytometric analysis of BrdU incorporation and DNA content of monocytes [gated as in **(B)**] 4 hours after intravenous (IV) injection of 5 μ g M-CSF and 1-hour BrdU labeling, showing representative profiles and the ratio of cycling to noncycling monocytes (WT $n = 3$, Maf-DKO $n = 2$). **(D)** Serial replating in methocult assay of monocyte-derived Maf-DKO cells washed out from M-CSF colony assays. **(E)** Growth curves of individual clones in liquid M-CSF culture. All colony assays show average CFU of replicate plates, all error bars indicate SEM, and **(A)**, **(B)**, and **(D)** are representative of at least two independent experiments.

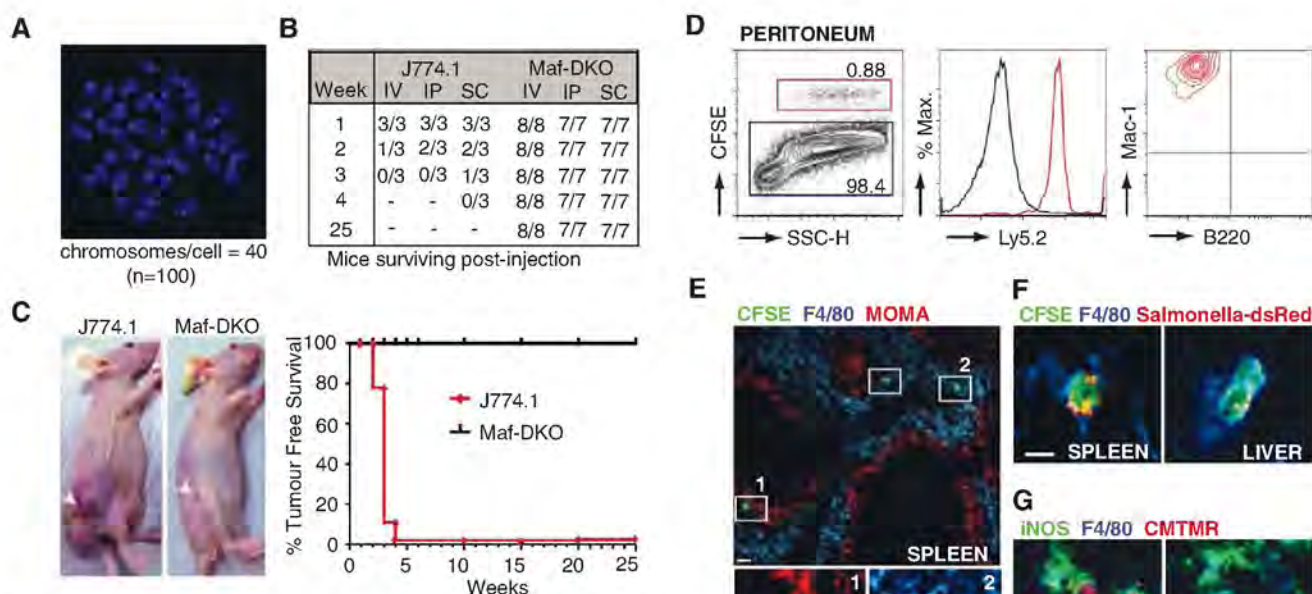
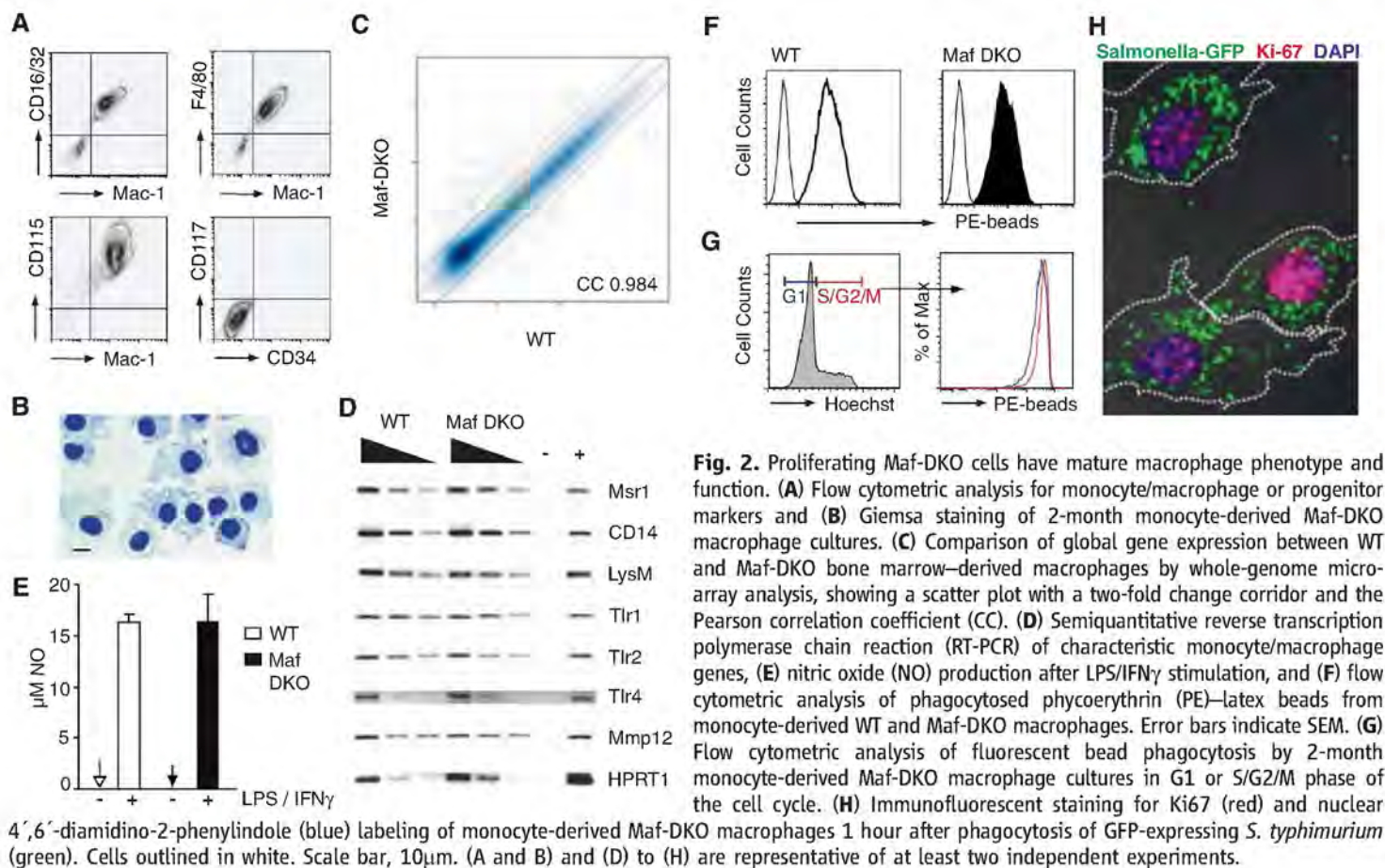
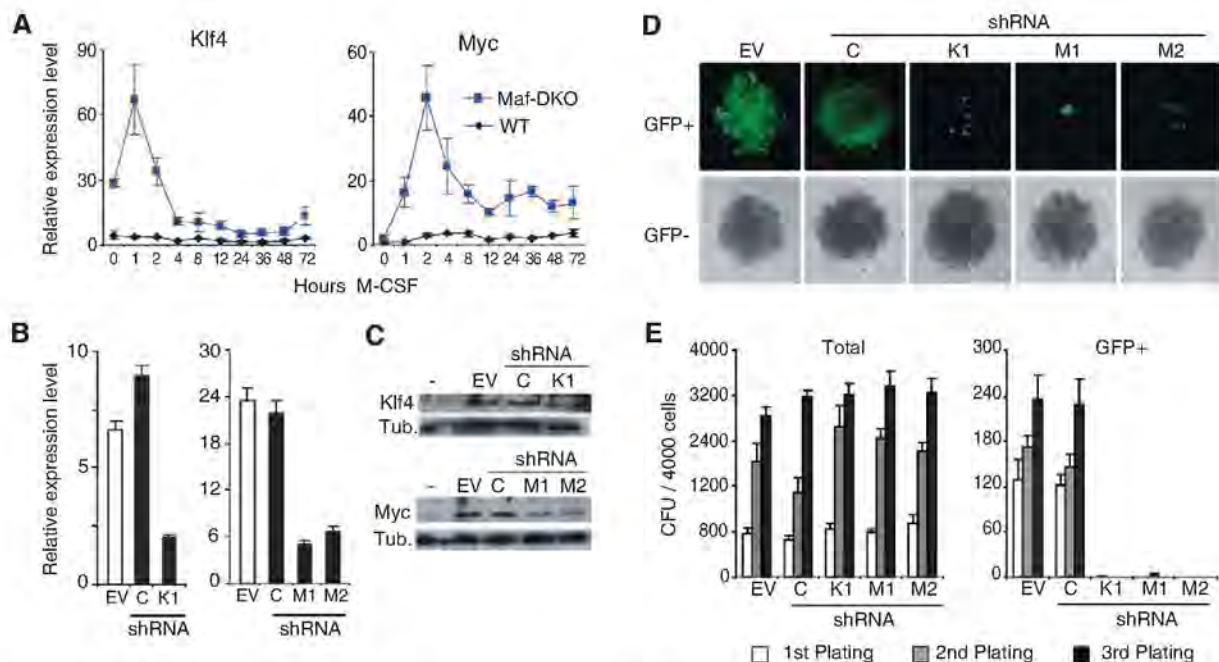


Fig. 3. Expanded Maf-DKO cells are not tumorigenic, but rather integrate as functional macrophages into host tissues in vivo. (A) Karyotype of long-term expanded monocyte-derived Maf-DKO macrophages. (B) Tumor-free survival of animals upon intravenous (IV), intraperitoneal (IP), or subcutaneous (SC) injection of 1×10^6 J774.1 or monocyte-derived Maf-DKO macrophages into syngeneic hosts. (C) Tumor development at 4 weeks and survival curve post-SC injection of 1×10^6 J774.1 or monocyte-derived Maf-DKO macrophages into nude mice ($n = 3$). Arrowheads indicate injection site. (D) Detection of transplanted carboxyfluorescein diacetate succinimidyl ester (CFSE)-labeled Ly5.2 $^{+}$ Maf-DKO macrophages by flow cytometric analysis of peritoneal exudate 6 days after IP injection and (E) by confocal immunofluorescence analysis of macrophage antigens on CFSE $^{+}$ cells in spleen 3 days after IV injection. Scale bars, 50 μ m (top), 20 μ m (bottom). Immunofluorescence analysis of CFSE-labeled (F) and 5-(and-6)-((4-chloromethyl)benzoyl)amino tetramethylrhodamine (CMTMR)-labeled (G) Maf-DKO macrophages for F4/80 (F) and (G) and iNOS expression (G) 3 days after transplantation and 48 hours after IV inoculation with the red fluorescent protein dsRed-expressing *S. typhimurium*. Scale bar, 20 μ m. (D) to (G) are representative of at least two independent experiments.

Fig. 4. Elevated Klf4 and c-Myc expression are both required for extended proliferation of Maf-DKO macrophages. **(A)** Quantitative RT-PCR analysis for Klf4 and c-Myc after M-CSF stimulation of cytokine-starved WT and Maf-DKO macrophages or **(B)** in NIH3T3 cells expressing empty vector (EV), control (C), Klf4 (K1) or c-Myc (M1 and M2) shRNA, respectively. **(C)** KLF4 and c-Myc immunoblot of QT6 cells cotransfected with empty (–), Klf4, or c-Myc expression vectors and control, Klf4 (K1), or c-Myc (M1 and M2) shRNA, as indicated. **(D)** Representative images of infected (GFP⁺) and uninfected (GFP[–]) colonies from the same M-CSF colony assays of monocyte-derived Maf-DKO macrophages transduced with the indicated shRNA-GFP retroviruses. **(E)** CFU counts for total (GFP⁺ and GFP[–]) and GFP⁺ colonies upon serial replating of the same assays. All error bars indicate SEM and all panels are representative of at least two independent experiments.



possibly unlimited, expansion of Maf-DKO cells could be achieved in liquid culture. We have maintained Maf-DKO cells in continuous culture for more than 8 months without any signs of crisis. For three independent Maf-DKO populations, cell counts over 2 months revealed stable doubling times of 1.44 ± 0.05 days and theoretical amplification factors of 10^{11} to 10^{12} (fig. S4A). This was unlikely to be due to the outgrowth or selection of a small subpopulation; more than 80% of Maf-DKO cells gave rise to new colonies in replating assays (Fig. 1D), and individual colonies could be cloned and subcloned at 80 to 90% efficiency (fig. S4B) or undergo expansion in liquid culture with similar, unaltered growth curves (Fig. 1E). Together, the high cloning and recloning efficiency and the similar growth rates of individual clones indicate that a vast majority, possibly all, rather than a subpopulation of Maf-DKO cells have an extended proliferation capacity.

In specialized regenerative processes, differentiated cells can also reenter the cell cycle, but in these examples cells typically undergo dedifferentiation and revert to an immature phenotype (3). By contrast, proliferating Maf-DKO cells maintained a differentiated phenotype and function. Like WT monocytes in M-CSF culture, Maf-DKO cells remained positive for the monocyte/macrophage surface markers FcγRII/III (CD16/32), Mac-1 (CD11b), F4/80, and CD115 (Fig. 2A); displayed a normal macrophage morphology (Fig. 2B); and were negative for progenitor markers CD117 and CD34 (Fig. 2A) or other lineage markers (fig. S5A), even after long-term expansion. Proliferating Maf-DKO cells also displayed a global gene expression profile highly similar to WT cells (Fig. 2C) and expressed a panel of characteristic monocyte/macrophage genes (Fig. 2D and fig. S5B). They

also showed the same capacity as WT monocyte-derived macrophages to produce nitric oxide in response to lipopolysaccharide and interferon- γ (Fig. 2E) and to phagocytose latex beads (Fig. 2F). Cell cycle analysis further demonstrated that Maf-DKO cells in S and G2/M phase had the same amount of phagocytic activity as cells in G0/G1 phase, indicating that differentiated macrophage function is fully maintained through cell cycle progression in these cells (Fig. 2G). This was further confirmed with live bacteria, revealing that cycling (Ki67⁺) Maf-DKO cells could phagocytose large numbers of green fluorescent protein (GFP)-expressing *Salmonella typhimurium* (Fig. 2H). Interestingly, M-CSF-expanded monocyte-derived Maf-DKO cells retained the ability to acquire dendritic cell features when shifted to GM-CSF-containing medium (fig. S6A and B).

To determine whether the extended proliferative capacity of Maf-DKO monocytes and macrophages was associated with tumorigenic transformation, we analyzed the long-term effects of MafB/c-Maf deficiency in vivo. Interestingly, bone marrow chimeras with a Maf-DKO hematopoietic system showed no sign of leukemia or myelo-proliferative disease for more than 1 year after reconstitution (fig. S7) or after hematopoietic stress induced by pharmacological hematopoietic stress (fig. S8). Furthermore, monocyte-derived Maf-DKO macrophage cultures retained a normal number of chromosomes through long-term ex vivo expansion (Fig. 3A) and did not give rise to tumors upon transplantation into syngeneic or immunocompromised nude mice, irrespective of the injection route (Fig. 3, B and C), despite the cells' ability to divide in vivo (fig. S9A). By comparison, under the same conditions the murine macrophage cell line J774.1 induced massive

tumors within days and caused 100% mortality by 4 weeks (Fig. 3, B and C).

Rather than forming tumors, transplanted Maf-DKO cells showed homing and functional integration into normal macrophage populations of multiple tissues. Maf-DKO cells contributed to macrophages of the bone marrow, peritoneum, and red pulp and marginal zone of the spleen, and to Kupffer cells of the liver (Fig. 3, D and E, and fig. S9, B and C). Moreover, Maf-DKO macrophages in spleen and liver participated in the characteristic host response to infection with live *S. typhimurium*, which causes an invasive disease in mice similar to human typhoid fever (14). The transplanted cells phagocytosed bacteria (Fig. 3F), localized to typical discrete macrophage foci in infected organs (14), and activated expression of inducible nitric oxide synthase (iNOS) (Fig. 3G and fig. S10). Furthermore, some transplanted cells acquired CD11c expression in infected mice (fig. S6C). Together, these results indicate that expanded Maf-DKO cells are not transformed but give rise to functional macrophages and possibly monocyte-derived dendritic cells that integrate into the normal tissue architecture in vivo.

The p19^{ARF}/p53 and p16/Rb cell cycle inhibitory pathways represent important control mechanisms of cellular proliferation, and their inactivation can extend the limited division number of mitotic progenitors in culture (15). However, neither mature leukocytes from p53^{−/−} nor those from p19^{ARF}/p16 (INK4a) double-deficient mice gave rise to M-CSF colonies (fig. S11), indicating that the observed self-renewal of differentiated Maf-DKO monocytes and macrophages must be based on distinct mechanisms.

Differentiated cells can be reprogrammed into pluripotent stem cells (iPS) by the four tran-

scription factors Oct-4, Sox-2, KLF4, and c-Myc (16, 17), of which the latter two have been proposed to impart extended proliferation capacity (16) on the basis of their role in embryonic stem cell self-renewal (18, 19). In addition, KLF4 and c-Myc can also mediate monocytic differentiation (20) and proliferation (21, 22), respectively. We therefore investigated the role of these factors in the extended proliferative capacity of Maf-DKO monocytes and macrophages. Whereas expanded Maf-DKO macrophage cultures did not express the pluripotency-associated factors Sox-2, Oct-4, and nanog (fig. S12A), they showed a strong up-regulation of both KLF4 and c-Myc expression within 2 hours of M-CSF stimulation and maintained substantially higher expression levels than WT controls for the observation period of 72 hours (Fig. 4A). To determine the functional consequence of these changes, we generated small hairpin RNA (shRNA) retroviral vectors directed against KLF4 or c-Myc that could specifically reduce both endogenous and transfected target gene expression at the RNA and protein level (Fig. 4, B and C). When monocyte-derived Maf-DKO macrophages were infected with GFP-expressing retrovirus coding for no or control shRNA sequences, they gave rise to GFP⁺ colonies in methocult assays of the same size and morphology as uninfected cells. By contrast, cells infected with GFP retrovirus expressing either KLF4 or c-Myc shRNA gave rise to only small GFP⁺ cell clusters of less than 20 cells (Fig. 4D) that could not be propagated through serial replating (Fig. 4E). Internal controls of noninfected GFP⁺ colonies from the same plating showed identical morphology, frequency, and replating behavior under all conditions (Fig. 4D). Conversely, combined ectopic overexpression of c-Myc and KLF4 conferred serial replating capacity to in vitro differentiated WT macrophages (fig. S12B). Together, these results indicated that increased expression of both KLF4 and c-Myc are required and potentially sufficient for the extended self-renewal capacity of Maf-DKO macrophages.

Together, our results indicate that MafB/c-Maf deficiency dissociates cell cycle exit from terminal macrophage differentiation. Although specialized mononuclear phagocytes such as microglia and some dendritic cells can undergo transient proliferation in peripheral tissues (23–25), their number of divisions is limited and it remains unresolved whether proliferation occurs in precursors or fully differentiated cells. Here, we show that MafB/c-Maf deficiency enables long-term expansion of differentiated, mature macrophages without loss of function or sensitivity to homeostatic control in vivo. Interestingly, this depends on the regulated activation of c-Myc and KLF4. Mechanistically, this may involve the loss of MafB/c-Maf mediated repression of Ets-1/2 (26) and PU.1 (7) (fig. S13), two myeloid activators of the *c-Myc* (4, 27) and *KLF4* (20) promoters, respectively. KLF and c-Myc are two iPS-inducing factors that have been proposed to mediate self-renewal (16, 18, 19) but are not required for pluripotency (17). Thus, it appears that pluripo-

tency and self-renewal are two stem cell characteristics that can be mechanistically dissociated. The nontumorigenicity of Maf-DKO macrophages is intriguing, given that individually both c-Myc and KLF4 can act as oncogenes (28, 29). In particular c-Myc can malignantly transform macrophages (21, 22) (fig. S12B) and induce tumors in iPS-derived mice (17). The controlled and joint up-regulation of c-Myc and KLF4 in Maf-DKO cells, however, may prevent malignancy (fig. S12B) by assuring a fine-tuned counterbalance of the factors' partially antagonistic activities in cell cycle control (16, 28). Together, our results provide a first example for extended amplification of functional differentiated cells without passing through pluripotent or multipotent stem cell intermediates and may open up new perspectives for monocyte-based cellular therapies in infectious disease or tissue regeneration.

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Materials and Methods
Figs. S1 to S13
Tables S1 and S2
References

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A Single Peptide–MHC Complex Positively Selects a Diverse and Specific CD8 T Cell Repertoire

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Pathogen recognition by T cells is dependent on their exquisite specificity for self–major histocompatibility complex (MHC) molecules presenting a bound peptide. Although this specificity results from positive and negative selection of developing T cells in the thymus, the relative contribution of these two processes remains controversial. To address the relation between the selecting peptide–MHC complex and the specificity of mature T cells, we generated transgenic mice that express a single peptide–MHC class I complex. We demonstrate that positive selection of CD8 T cells in these mice results in an MHC-specific repertoire. Although selection on a single complex is peptide promiscuous, mature T cells are highly peptide specific. Thus, positive selection imparts MHC and peptide specificity on the peripheral CD8 T cell repertoire.

In the thymus, through the processes of positive and negative selection, T cells develop the ability to respond to pathogen- or tumor-derived peptides presented by major histocom-

patibility complex (MHC) but remain tolerant to MHC presenting self-peptides. Positive selection results from low-avidity interactions between the T cell receptor (TCR) and peptide–MHC during

thymic development, producing T cells that can recognize self-MHC-presented foreign peptide. Negative selection deletes T cells with high avid-

ity for self-peptide-MHC. Although peptide was implicated in positive selection of CD8 T cells in vitro (1–6), the extent to which it contributes to the specificity of mature CD8 T cells and the structural relation between the selecting and activating peptides have not been defined in vivo. To address these issues, we generated transgenic mice that express a single peptide-MHC class I (MHCI) complex.

MHCI molecules were engineered as single-chain trimers (SCTs) in which peptide, β_2 -

microglobulin (β_2M), and the MHCI α chain are covalently attached through flexible linkers (fig. S1). SCTs are efficiently expressed, exceptionally stable at the cell surface, resistant to peptide exchange, and retain their native structure required to potently stimulate T cells (7–9). To study CD8 T cell development, we generated transgenic mice with an MHCI promoter driving the expression of SCTs based on the H2-K^b MHCI α chain, and peptides derived from chick-

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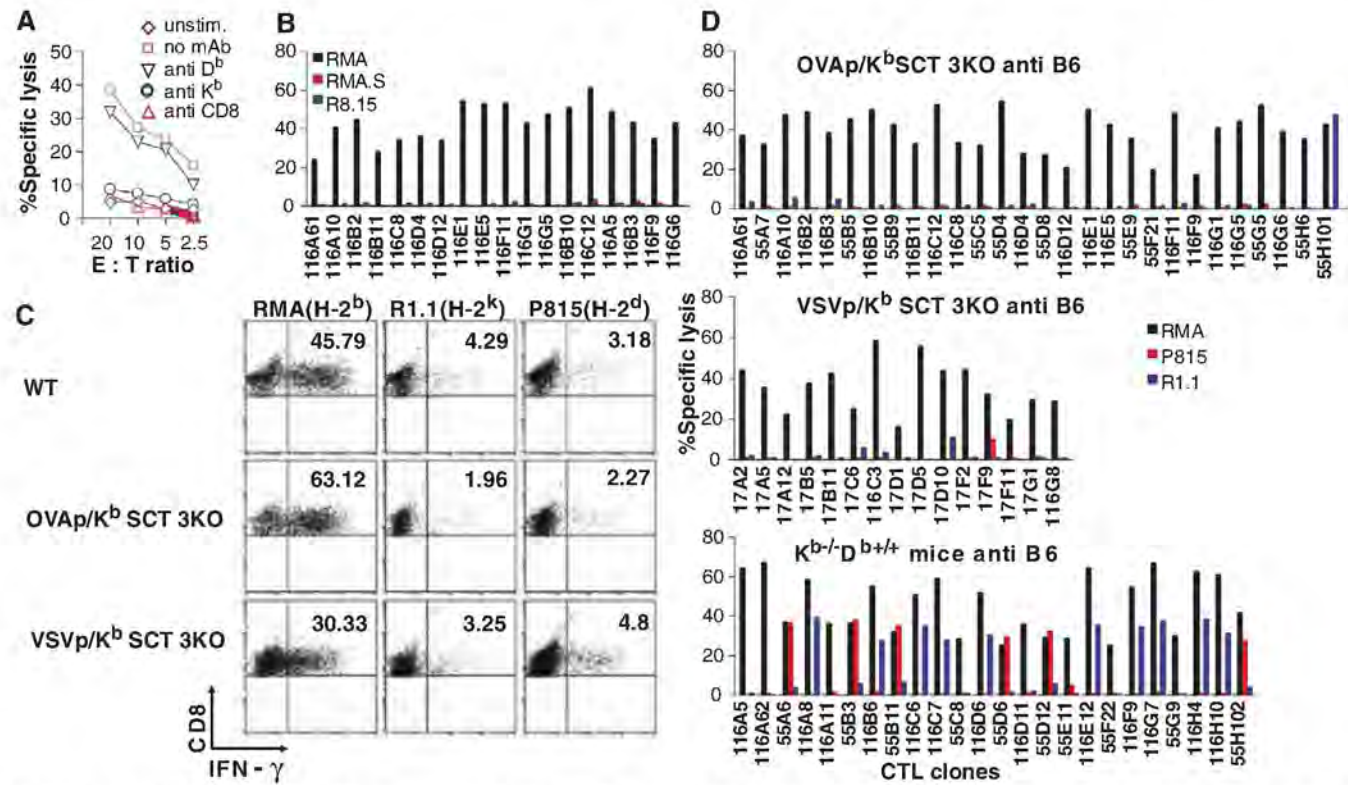
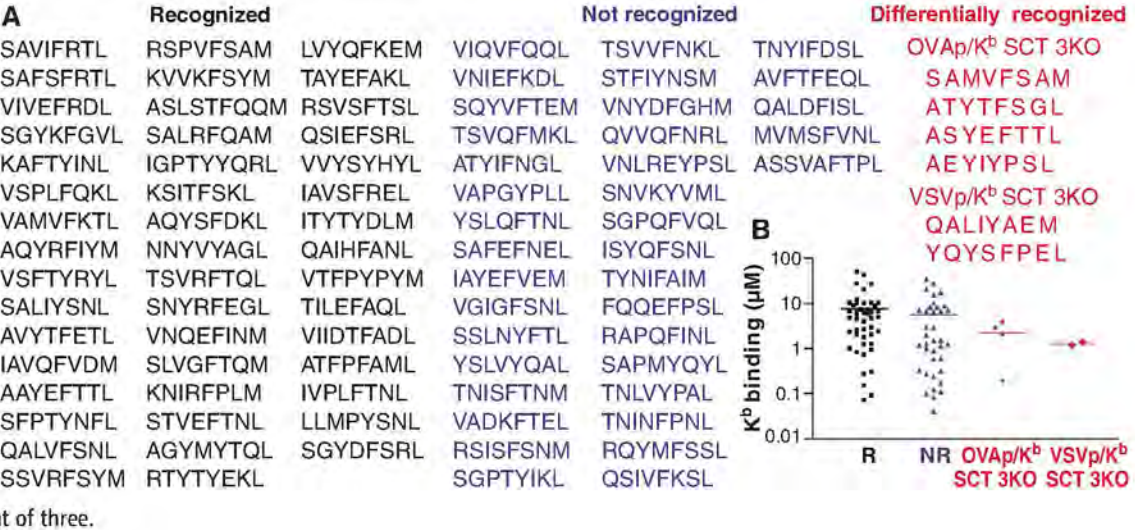


Fig. 1. SCT 3KO primary CTLs are K^b specific and non-MHC cross-reactive. (A) OVAp/K^b SCT 3KO splenocytes stimulated with B6 splenocytes were cultured with RMA (H2^b) target cells in the absence or presence of monoclonal antibody. Data represent one experiment of six. (B) OVAp/K^b SCT 3KO anti-B6 CTL clones were cultured with RMA (K^bD^b), RMA.S (TAP^{−/−}, H2^b), or R8.15 (K^bD^b) target cells. Data represent one experiment of three. (C) K^b-reactive CTLs generated from control K^bD^b+/+ splenocytes and SCT 3KO splenocytes,

stimulated with K^b+/+D^b−/− splenocytes, were replated with the indicated stimulator cells. Production of intracellular interferon- γ (IFN- γ) by CD8⁺ T cells was measured by flow cytometry. Data represent one experiment of three. (D) CTL clones were generated from SCT 3KO mice and K^b−/−D^b+/+ mice stimulated with B6. The CTL clones were cultured with RMA (H2^b), P815 (H2^d), or R1.1 (H2^k) target cells. Data are from one representative experiment. Clones were assayed two to four times.

Fig. 2. Recognition by SCT 3KO mice of K^b endogenous peptides complexed with H2-K^b. (A) The sequences of the 90 K^b-binding peptides (10) are listed on the basis of their ability to stimulate polyclonal T cells from the two strains of SCT 3KO mice. Peptides were assayed at least three times. (B) K^b binding was measured by means of an MHC class I surface induction assay (30). Binding is plotted as the concentration of peptide that resulted in a threefold increase in the expression level of K^b on RMA.S cells. The bar indicates the mean peptide concentration. Data represent one experiment of three.



en ovalbumin [SIINFEKL (10), OVAp] or vesicular stomatitis virus (RGYVYQGL, VSVp). We bred these mice to B6 mice deficient in β_2M and MHC I molecules K^b and D^b (3KO) to obtain mice that express only the SCT and not other MHC I molecules (SCT 3KO) (fig. S2A). With this system, we analyzed the *in vivo* development of CD8 T cells in the presence of a single peptide-MHCI complex.

To assess CD8 T cell development in SCT 3KO mice, we analyzed CD8 and CD4 expression on thymocytes and splenocytes (fig. S3). In the control 3KO mice, there were very few splenic CD8 T cells ($0.26 \pm 0.14\%$), consistent with their deficient expression of MHC I. The number of splenic CD8 T cells in both OVAp/ K^b and VSVp/ K^b SCT 3KO mice was markedly reduced ($4.2 \pm 0.9\%$) relative to the number in wild-type mice ($36.1 \pm 3.5\%$). These results differ from studies of CD4 T cell development in MHC class II single complex mice, where the number of splenic CD4 T cells is 20 to 40% of wild-type levels (11–13). The reduced frequency of CD8 T cells in SCT 3KO mice is consistent with positive selection being peptide specific because expression of SCTs is comparable to that

of native K^b on splenocytes and cortical thymic epithelial cells in wild-type mice (fig. S2, B and C). If positive selection required only the presence of peptide and not a specific peptide, then normal numbers of CD8 T cells would be expected. Despite the substantial reduction in CD8 T cells, TCR diversity (TCR V_β usage) did not differ from that of wild-type mice (fig. S4), similar to MHC class II single complex mice as well as mice deficient in β_2M , MHC I, or transporter associated with antigen processing (TAP) (12–16).

The CD8 T cells selected by SCT 3KO mice were highly reactive when stimulated with wild-type B6 ($H2-K^bD^b$) cells, as measured by cytotoxic T lymphocyte (CTL)-mediated lysis of labeled target cells (Fig. 1A). This robust response permitted us to test rigorously the MHC and peptide specificity of SCT-selected CD8 T cells. It is possible that the absence of negative selection on multiple MHC I alleles would result in highly MHC cross-reactive CD8 T cells. If so, then a large percentage would be reactive with both K^b and D^b antigens. However, SCT-selected CD8 T cells are almost exclusively K^b -reactive as demonstrated by antibody blocking (Fig. 1A) and selective lysis of K^b -expressing

targets (Fig. 1B). The preferential K^b reactivity of SCT-selected CD8 T cells supports the notion that MHC restriction is based on self-recognition during positive selection (17). Notably, negative selection occurs normally in SCT 3KO mice because T cells from these mice did not react to self-peptide-MHCI complexes (fig. S5).

We further analyzed for MHC cross-reactivity with $H2^k$ and $H2^d$ alleles. Polyclonal CD8 T cells from control (K^bD^b) and SCT 3KO mice stimulated with K^bD^b splenocytes exhibited low MHC cross-reactivity (Fig. 1C). Furthermore, K^b -reactive T cell clones from SCT 3KO mice were not more MHC cross-reactive on $H2^d$ and $H2^k$ alloantigens than clones generated from control (K^bD^b) mice (Fig. 1D). We conclude that SCT 3KO CD8 T cells are highly MHC specific.

The observation that single peptide-selected cells were highly reactive with K^b plus endogenous peptides demonstrated that SCTs exclude thymic presentation of endogenous K^b ligands, and permitted assessment of the peptide specificity of SCT 3KO T cells. To this end, we identified an extensive panel of naturally processed, K^b -binding peptides by using high-performance liquid chromatography fractionation and mass spectrometry (MS). Ninety peptides were selected for analysis based on their K^b -binding motif and MS confidence score from a protein database search (Fig. 2A). We generated polyclonal SCT 3KO CTLs and tested them by stimulating the CTLs with all 90 peptides individually with $TAP^{-/-}$ splenocytes as antigen-presenting cells and peptide-treated $TAP^{-/-}$ tumor target cells. Forty-seven peptides were recognized by both strains of SCT 3KO mice, 37 peptides were not recognized by either strain, and 6 were recognized by one or the other (differentially recognized) (Fig. 2A). We identified one peptide (RTYTYEKL) shown to be a selecting peptide with structural homology to the agonist peptide, OVA (18). However, computational analyses using Weblogo (19) identified no overall pattern of similarity between the peptide sequences within the groups, consistent with studies demonstrating that selecting and agonist peptides do not have to be structurally related (20, 21). The pronounced lack of sequence homology between the selecting and antigenic peptides is consistent with positive selection based on low-avidity TCR/peptide-MHC interactions, and TCR cross-reactions are difficult to predict on the basis of peptide sequences alone or known crystal structures of peptide-MHC complexes.

Several of the peptides stimulated CD8 T cells from both OVAp/ K^b and VSVp/ K^b SCT 3KO mice, raising the possibility that these T cells are degenerate in their peptide recognition. To test this, we selected six individual peptides that strongly activated CTLs from both strains of SCT 3KO mice. We generated primary CTLs from OVAp/ K^b SCT 3KO using these peptides, then tested the CTLs for cross-reaction to 18 other strongly recognized peptides. Polyclonal T

Fig. 3. SCT-selected cells are highly peptide specific. Splenocytes from OVAp/ K^b SCT 3KO mice were stimulated with six different peptides, indicated at the top of each panel, and tested with the stimulating peptide and 18 other peptides as indicated (10). Data represent one experiment of three.

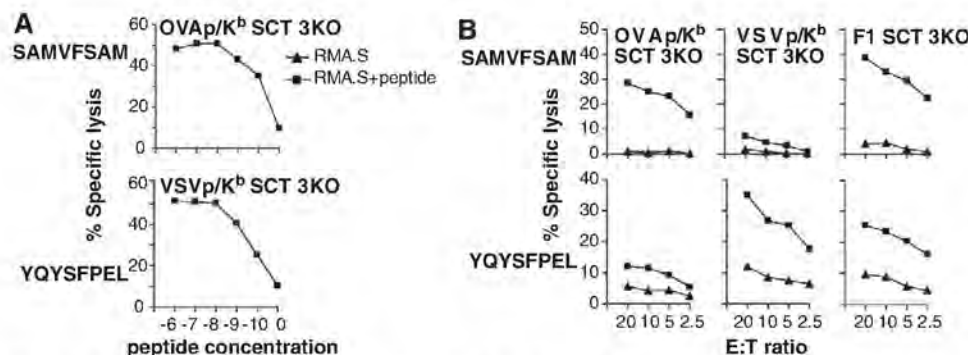
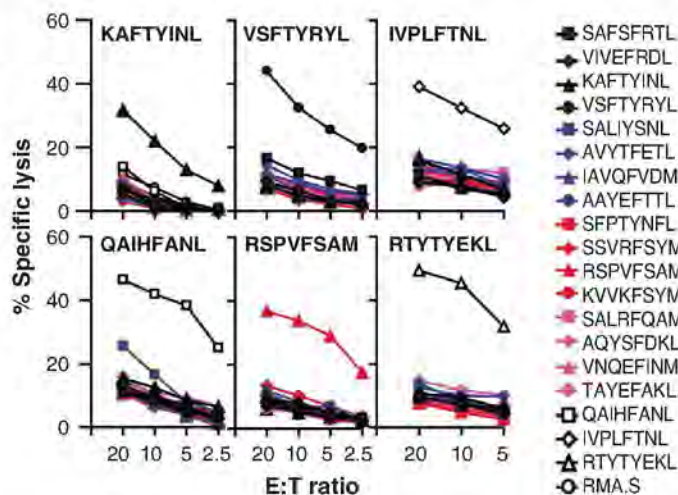


Fig. 4. T cell responses to differentially recognized peptides (10). (A) Primary SCT 3KO CTLs, stimulated with peptides recognized exclusively by OVAp/ K^b (SAMVFSAM) or VSVp/ K^b (YQYSFPEL), were tested for reactivity over a range of peptide concentrations. Data represent one experiment of three. (B) The same peptides in (A) were used to generate CTLs from OVAp/ K^b SCT 3KO, VSVp/ K^b SCT 3KO, and (OVAp/ K^b $\times$ VSVp/ K^b) F1 SCT 3KO mice and were tested for lysis of peptide-treated RMA.S target cells. Data represent one experiment of three.

cells stimulated with each of the peptides were highly peptide specific, with minimal cross-reaction (Fig. 3). Thus, CD8 T cells selected by the SCT are highly peptide specific.

The question remained why a high percentage of the peptides are recognized by both strains or neither strain of SCT 3KO mice. If the recognition were of contaminating peptides, then responsiveness to the peptides would correlate with affinity of the peptide for K^b. However, the activating and nonactivating peptides displayed a similar range of relative binding affinities (Fig. 2B). Furthermore, the evidence strongly indicates that the SCT efficiently excludes exogenous peptides (7). We conclude that the response to common peptides is the result of promiscuity during selection; i.e., a single peptide-MHC complex can select T cells specific for several different peptides, consistent with some (6, 13, 21, 22) but not other (23) studies. Furthermore, two unrelated peptide-MHC complexes can select T cell repertoires with overlapping specificities.

The differentially recognized peptides (Fig. 2A) were further analyzed to determine if disparities in recognition were absolute or due to differences in TCR avidity. The SCT-selected T cells that recognized the peptides did so with high avidity, whereas the same peptide was not recognized by the other SCT-selected T cells, even at high peptide concentrations and at several effector-to-target ratios (Fig. 4). Thus, the difference in peptide recognition between the two strains of mice is absolute. Accordingly, all six of the differentially recognized peptides were stimulatory to T cells from the (OVAp/K^b × VSVp/K^b) F₁ SCT 3KO mice (Fig. 4B and fig. S6), indicating that the response to these peptides was due to positive and not negative selection.

In sum, we provide direct *in vivo* evidence that the MHC and peptide specificity of the CD8 T cell repertoire is imparted by positive selection. Studies of mice expressing a single peptide-class II complex (11, 13, 23–26) arrived at conflicting conclusions regarding the role of positive versus

negative selection in determining the specificity of the CD4 T cell repertoire (23, 25, 26). Our results contrast with the report of Huseby *et al.*, which found that CD4 T cells selected on single peptide-class II complex mice are highly MHC and peptide degenerate and concluded that MHC and peptide specificity result from negative selection (26). It is unclear if these differences are due to the approaches used or disparities in how CD4 versus CD8 T cells are selected (27).

Studies using fetal thymic organ culture identified exogenous peptides capable of positive selection *in vitro* (1–6). This led to different theories regarding the nature of the selecting peptide and its relation to the agonist ligand (2, 4). In our *in vivo* repertoire analysis, positive selection of CD8 T cells is peptide promiscuous in that a single peptide-MHCI complex can select T cells with disparate peptide specificities, yet once selected the T cells are highly peptide specific. Furthermore, we show that two different peptide-MHCI complexes select T cells with both overlapping and unique peptide specificities and that there is no structural homology between selecting and agonist peptides. In contrast to the promiscuity of peptide specificity, MHC specificity imparted by positive selection was pronounced as indicated by the predominance of K^b-restricted T cells after SCT selection. Notably, once selected, all mature CD8 T cells are highly MHC and peptide specific, consistent with both being inherent properties of TCR/peptide-MHC interactions (28, 29).

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10. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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31. We thank P. Allen, C.-S. Hsieh, Y. Huang, K. Murphy, E. Unanue, and A. Singer for critical comments, and Y. Y. Yu for making the transgene constructs. Mass spectrometry was performed at the Washington University National Center for Research Resources MS Resource (grant 2P41RR000954). This work was supported by NIH grants AI 027568 and AI 055849.

Supporting Online Material

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Materials and Methods

Figs. S1 to S6

References

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Peter C. Agre, M.D.
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Dear Colleagues,

On behalf of the AAAS Board of Directors, it is my distinct honor to invite you to the 176th Meeting of the American Association for the Advancement of Science (AAAS).

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The Annual Meeting reflects contributions from the AAAS sections, which I gratefully acknowledge. I also extend a personal thanks to the Scientific Program Committee for assembling this outstanding meeting and to our local co-chairs, Marye Anne Fox, chancellor, University of California, San Diego, and Irwin Jacobs, co-founder, Qualcomm Inc.

I look forward to seeing you in San Diego,

Dr. Peter Agre, AAAS President and
Director, Malaria Research Institute,
Johns Hopkins Bloomberg School of Public Health

President's Address



Peter C. Agre, M.D.

AAAS President, and Director, Malaria Research Institute, Johns Hopkins Bloomberg School of Public Health

Agre shared the 2003 Nobel Prize in Chemistry with Roderick MacKinnon of Rockefeller University for the discovery of aquaporins, the key proteins that transport water across cell membranes.

Not long after receiving the Nobel Prize, Agre began working to extend his studies of aquaporins to malaria, addressing the question of whether or not aquaporins could be exploited as a means of treating or preventing the disease. Initial results led his laboratory to focus on malaria as its primary area of study. As director of the Malaria Research Center, he oversees 19 Hopkins faculty members who concentrate on advancing basic science to develop new methods in malaria prevention and treatment. Agre is a member of the National Academy of Sciences (NAS), chair of the NAS Committee on Human Rights, and a Fellow of AAAS and the American Academy of Arts and Sciences. He received his B.A. degree in chemistry from Augsburg College and his M.D. degree from Johns Hopkins University.

President's Reception: Immediately following

Plenary Speakers



Carol W. Greider, Ph.D.

Daniel Nathans Professor and Director, Department of Molecular Biology and Genetics, and Professor of Oncology, Johns Hopkins University School of Medicine, Baltimore, MD

Title To Be Determined

Greider, one of the world's pioneering researchers on the structure of telomeres, was awarded the 2009 Nobel Prize in physiology or medicine by the Royal Swedish Academy of Sciences along with Elizabeth Blackburn and Jack W. Szostak. While a 23-year-old graduate student at the University of California, Berkeley, working together with Blackburn, Greider discovered the enzyme telomerase and later, in her own lab, she cloned its RNA component. This work laid the foundation for studies that have linked telomerase and telomeres to human cancer and age-related degenerative disease. It represents another example of curiosity-driven basic research that has direct medical implications. Greider obtained her Ph.D. degree in molecular biology from UC Berkeley in 1987. She then went to Cold Spring Harbor Laboratory where she ran a lab for 10 years studying telomerase. In 1997 she joined the department of molecular biology and genetics at Johns Hopkins University School of Medicine in Baltimore. Greider grew up in Davis, Calif., where her father was a physicist at the University of California. She credits her father for encouraging her to pursue what most excited her.



Eric S. Lander, Ph.D.

Director, The Broad Institute of MIT and Harvard University, and Co-Chair, President's Council of Advisors on Science and Technology (PCAST) *Science and Technology in the First Year of the New Administration*

Lander is widely known as one of the driving forces behind today's revolution in genomics, the study of all of the genes in an organism and how they function together in health and disease. He also is co-chair of President Obama's council of science and technology advisers. PCAST is an advisory group of the nation's leading scientists and engineers who directly advise the President and make policy recommendations in the many areas where understanding of science, technology, and innovation is key to strengthening the economy and forming policy. Lander also was one of the principal leaders of the Human Genome Project and is a member of both the National Academy of Sciences and Institute of Medicine. He is also an AAAS Fellow. Lander earned his B.A. degree in mathematics from Princeton University and Ph.D. degree in mathematics from Oxford University as a Rhodes Scholar. He also was an assistant and associate professor of managerial economics at the Harvard Business School.



Marcia McNutt, Ph.D.

Director, U.S. Geological Survey, and Science Adviser to the Secretary, U.S. Department of the Interior (*invited*)

Title To Be Determined

McNutt's appointment in 2009 marked a milestone for USGS -- she is the first female director in the agency's 130-year history. She directs a multi-disciplinary organization that focuses on biology, geography, geology, geospatial information, and water, and is dedicated to studying the landscape, natural resources, and natural hazards. Most recently she served as president and chief executive officer of the Monterey Bay Aquarium Research Institute. Her biography includes a broad range of research interests and numerous honors and awards. She has participated in 15 major oceanographic expeditions and served as chief scientist on more than half of them. Her research has ranged from studies of ocean island volcanism in French Polynesia to continental break-up in the Western United States to uplift of the Tibet Plateau. McNutt studied geophysics at the Scripps Institution of Oceanography and earned her Ph.D. degree there in earth sciences in 1978. She then spent 3 years with the USGS in California working on earthquake prediction. At MIT she was appointed the Griswold Professor of Geophysics and served as director of the Joint Program in Oceanography and Applied Ocean Science and Engineering. She is a member of the National Academy of Sciences and the American Academy of Arts and Sciences, and a Fellow of AAAS, American Geophysical Union, and Geological Society of America.

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The Future of Stem Cell Research

Thomas Hillman Jordan

Director, Southern California Earthquake Center, and the W. M. Keck Professor of Earth Sciences, University of Southern California
Understanding Earthquakes Through Large-Scale Simulations

Stephen R. Palumbi

Professor of Biological Sciences, Stanford University
How Marine Species React and Adjust to Ocean Acidification and Climate Change

Kellogg Schwab

Associate Professor and Director of the Center for Water and Health, Johns Hopkins Bloomberg School of Public Health
Improving Access to Potable Water Throughout The World

Steffanie Strathdee

Associate Dean of Global Health Sciences, Harold Simon Professor, and Chief of the Division of Global Public Health, School of Medicine, University of California, San Diego
Infectious Diseases Have No Passport: Battling HIV, TB, and STDs on the Mexico-U.S. Border

2010 GEORGE SARTON MEMORIAL LECTURE

Jed Z. Buchwald

Doris and Henry Dreyfuss Professor of History, California Institute of Technology
Knowledge in the Early Modern Era: The Origins of Experimental Error

Seminar Tracks

Day-long seminars address topics that build bridges between science and society.

Translational and Personalized Medicine

Friday, 19 February

Translational research transforms scientific discoveries from the laboratory bench into practical clinical applications at the patient's bedside. This seminar focuses on the challenges and opportunities in translating the burgeoning science and technology of genomics into a greater understanding of human diseases and personalized treatment.

Evaluating and Funding Translational Research

Organized in cooperation with the journal, Science Translational Medicine

During the last 10 years a new breed of scientist, the clinician-researcher, evolved to take on translational medicine challenges, and a novel set of review criteria for interdisciplinary projects was developed by funding agencies to stimulate translational research. This session addresses the criteria for evaluating translational research and the effectiveness of the NIH Roadmap and the FDA Critical Path Initiatives. It will also cover new paradigms for drug development with an emphasis on biomarker and imaging endpoints. Finally, the speakers will attempt to glance at the future, predicting where translational medicine might be in 2020.

Organized by Maria T. Vassileva, Foundation for the National Institutes of Health; Juli Staiano, AAAS Development; Katrina Kelner, Science Translational Medicine

SPEAKERS

Eric J. Topol, The Scripps Research Institute, La Jolla, CA

Outcomes of the NIH Roadmap: Impact on Translational Medicine

Gail Cassell, Eli Lilly and Co., Indianapolis, IN
The FDA Critical Path Initiative: A Perspective of the First Four Years

Ellen V. Sigal, Friends of Cancer Research, Arlington, VA

Novel Funding Models for Translational Research

DISCUSSANT

Gary Firestein, University of California, San Diego

Genome Analyses and Sequencing To Advance Drug Discovery and Treatment

This session will address the rapidly moving scientific and technological field of genomics and its utility in elucidating the biological basis of human diseases and developing molecular diagnostics that can be used to individualize drug therapy of human diseases. Topics will cover whole-genome sequencing using "next generation" technologies, genome wide SNP analyses, and candidate gene strategies as well as the computational, ethical, and privacy issues surrounding the generation of whole genome data for individual patients. This field of science and technology will touch every area of health care in the coming decade.

Organized by William Evans, St. Jude Children's Research Hospital

SPEAKERS

Richard Wilson, Washington University School of Medicine, St. Louis

Discovery of Poly-Genetic Determinants of Diseases Through Whole Genome Sequencing
Mary Relling, St. Jude Children's Research Hospital, Memphis, TN

Genetics of Racial Differences in Drug Response, Disease Risk, and Health Disparities

Dan Roden, Vanderbilt University School of Medicine, Nashville, TN

Challenges and Opportunities in the Assembly of Population Pharmacogenomics

DISCUSSANTS

Scott Weiss, Harvard Medical School, Boston, MA
William Evans, St. Jude Children's Research Hospital, Memphis, TN

The Road to Personalized Medicine

The goal of personalized medicine is to deliver the right drug to the right person at the right dose in an effort to reduce the cost of high quality health care and minimize human suffering due to adverse events. For example, hospitalization due to adverse drug reactions in Britain alone is estimated to cost \$847 million annually. New tools are required to improve decision-making during drug development and in the clinic. This session will provide insight into technological advancements spurring the discovery of new biomarkers such as genes, proteins, and metabolites. Translational approaches to biomarker identification and qualifications for use will be presented. Experiences with human clinical trials that implement pharmacogenetic biomarkers of safety and efficacy will be described.

Organized by Donna L. Mendrick, U.S. Food and Drug Administration; Vishal S. Vaidya, Harvard Medical School

SPEAKERS

Ivan Rusyn, University of North Carolina, Chapel Hill

Modeling Toxicity in the Population Using Experimental Models

Vishal S. Vaidya, Harvard Medical School, Boston, MA

Bench to Bedside Detection of Kidney Toxicity

Maryellen de Mars, Critical Path Institute, Tucson, AZ

Using Genetic Information To Predict and Prevent Drug Toxicity

DISCUSSANT

Donna L. Mendrick, U.S. Food and Drug Administration, Silver Spring, MD

Marine Sciences and Society

Saturday, 20 February

The oceans provide us with many economic and aesthetic benefits as well as vital ecosystem services. These include seafood, pharmaceuticals, minerals, recreation, and much of the oxygen we breathe. Evaluating available science and unique aspects of marine systems is critical to successful ocean stewardship.

Does Size Matter? Rationales for Large Marine Reserves

Research has demonstrated the value of the world's great terrestrial parks, from Yellowstone to the Serengeti, in preserving ecosystems, protecting wide-ranging species, and supporting non-extractive industries. Do large ocean reserves offer similar benefits? This session will examine the successes of large terrestrial parks, compare marine and terrestrial reserves of similar scale, and explore conservation benefits of large marine reserves, including increased resilience to climate change. Speakers will discuss what is being learned from existing large, no-take marine reserves and consider ongoing and potential efforts to establish additional large protected areas.

Organized by Emily Frost and Angela T. Bednarek, The Pew Charitable Trusts; Terry Hughes, James Cook University, Australia

MODERATOR

Jane Lubchenco, National Oceanic and Atmospheric Administration, Washington, DC

SPEAKERS

Stuart L. Pimm, Duke University, Durham, NC
Large Terrestrial Protected Areas and Lessons for the Marine Environment

Stephen R. Palumbi, Stanford University, CA
Spreading the Wealth: Design and Function of Highly Protected Reserve Networks

Terry Hughes, James Cook University, Townsville, Australia

Proving the Benefits of Very Large Marine Reserves

DISCUSSANT

Jay Nelson, The Pew Charitable Trusts, Philadelphia, PA

Marine Spatial Planning: A New Approach for Balancing Ocean Uses and Ecosystem Health

Human activities have led to significant degradation of ocean ecosystems. This failure is largely due to a traditionally balkanized approach to ocean management. Each activity—such as fishing, shipping, oil and gas development, and renewable energy production—is governed by a different set of laws and administered by a different agency. President Obama has directed all federal agencies with jurisdiction over activities that affect the oceans to develop a “framework for effective coastal and marine spatial planning” that “addresses conservation, economic activity, user conflict, and sustainable use of ocean, coastal, and Great Lakes resources.” This symposium explores the latest developments in the science, policy, and practice of marine spatial planning.

Organized by Morgan Gopnik, Nicholas Institute for Environmental Policy Solutions; Mary Turnipseed, Duke University

SPEAKERS

Larry Crowder, Duke University, Beaufort, NC

The Science and Management of Coupled Social-Ecological Systems in the Ocean

Kevin St. Martin, Rutgers, The State University of New Jersey, New Brunswick

Mapping Communities: Linking People to Ocean Spaces

Andrew Rosenberg, University of New Hampshire, Durham

Advancing Ocean Planning in Massachusetts: The Role of a Unique Stakeholder Coalition

Mary Turnipseed, Duke University, Durham, NC

Re-Imagining the Public Trust Doctrine To Inform U.S. Marine Spatial Planning

Jo Foden, University of East Anglia, Norwich, United Kingdom

Evaluating Marine Plans: Lessons Learned from Aquatic Environmental Assessments

Fanny Douvère, UNESCO, Paris

Marine Spatial Planning: A Step-by-Step Approach Toward Ecosystem-Based Management

Arctic Sea-Ice Loss: What This Means for the Conservation of Arctic Marine Ecosystems

Sea-ice, a distinguishing feature of polar oceans, has a significant influence on the life history, diet, and general ecology of polar marine organisms. Present-day sea-ice loss is fundamentally altering the structure and function of the various components of marine ecosystems in the Arctic,

from primary producers to top predators. The observed and projected reduction in perennial sea-ice coverage also will leave room for increased human activity such as transportation, commercial fishing, and oil and gas exploration. To be effective, appropriate management and conservation of the Arctic Ocean must include the potential future response of Arctic ecosystems to sea-ice melt due to climate forcing. This session will explore sea-ice variability in a melting Arctic, offer background on the linkages between sea-ice and Arctic marine ecosystems, examine how they may be responding to reduced ice coverage, and discuss the data and steps that are needed for an effective Arctic conservation plan.

Organized by Tara Connelly and Gabriela Chavarria, Natural Resources Defense Council

SPEAKERS

John Walsh, University of Alaska, Fairbanks
Climate Change in the Arctic: What Are the Signs and What Is Predicted?

Jacqueline Grebmeier, University of Tennessee, Knoxville

The Potential Effect of Sea-Ice Loss on Arctic Marine Ecosystems

Frances Beinecke, Natural Resources Defense Council, Washington, DC

Role of the Aspen Institute's Commission on Arctic Climate Change in the Arctic

DISCUSSANT

Charles Clusen, Natural Resources Defense Council, Washington, DC

History and Future of Laser Technology

Sunday, 21 February

A prominent example of the impact that pure scientific research can have on society is the story of the laser. The 50th anniversary of the first working laser takes place in 2010. From DVD players to eye surgery, the laser is one of the greatest inventions of the 20th century and has revolutionized daily life.

Celebrating the Birth of the Laser: A Look Back After 50 Years

In 1960, the laser was an embryonic research tool with no clear applications beyond the laboratory—“a solution in search of a problem.” Since then, the laser has acquired immense commercial, industrial, and scientific importance. Its impact on how we live, from health care to entertainment to national security, has been enormous. This session tells the story of how the laser came to be, and provides a first-hand account of the birth and early growth of this ubiquitous scientific device.

Organized by Alan Chodos, American Physical Society, College Park, MD; Anthony J. Campillo, Optical Society of America, Washington, DC

SPEAKERS

Anthony Siegman, Stanford University, CA
How the Laser Came To Be
William B. Bridges, California Institute of Technology, Pasadena
Gas Lasers: The Early Years
Jeff Hecht, *Laser Focus World*, Auburndale, MA
Looking Back at How the Laser Evolved

Next Generation of Extreme Optical Tools and Applications

The "Century of the Photon" began in 1960 with the invention of a unique light source, the laser. With advancements in higher power, narrower color, shorter wavelength, or higher quality, the usefulness of light as a probe has increased. Each of the laser-based tools covered in this session extends the utility of the photon as a probe of the fundamental properties of the universe and represents the current state-of-the-art in developing the next generation of measurement tools and techniques. Topics include the generation of light at the extremes of intensity, photon energy, pulse duration, brightness, and power as well as new applications. Finally, new tools may lead to optical probing of the weakest of the forces, gravity.

Organized by Christopher Ebberts, Lawrence Livermore National Laboratory

SPEAKERS

Robert L. Byer, Stanford University, CA
Quantum Noise Limited Lasers and the Search for Gravitational Waves
Margaret Murnane, University of Colorado, Boulder
Attosecond Light and Science at the Time-Scale of Electron Motion
Christopher Barty, Lawrence Livermore National Laboratory, Livermore, CA
Revolutionizing Isotope Science and Applications with Laser-Like Gamma-Rays
Keith Hodgson, SLAC National Accelerator Laboratory, Menlo Park, CA
Next Generation X-Ray Lasers and Applications

Toshiki Tajima, Max Planck Institute for Quantum Optics, Garching, Germany
Relativistic Optics and Applications with Ultra-Intense Lasers
Wim Leemans, Lawrence Berkeley National Laboratory, Livermore, CA
Laser-Based Particle Acceleration and the Path to TeV Physics

Lasers at the Extreme: Ultra-Cold, Ultra-Fast, and Ultra-Hot Uses

Lasers at the extreme dominate this session. Lasers are involved with the cooling and trapping of atoms, generating "ultra-cold" states of matter on Earth—less than a billionth of a degree above Absolute Zero—that allow the testing of assemblies of atoms governed by quantum mechanical principles. Fiber-optic-based data communication—laser beams transmitted through glass wires—now dominate the movement of information, transmitting much of the world's conversations and commerce at "ultra-fast" data rates. The National Ignition Facility is now fully operational and ready to conduct experiments at the extremes of pressure and temperature. The experimental campaign to create "ultra-hot" matter in a laboratory, previously occurring only deep within the center of stars, is upon us.

Organized by Thomas M. Baer, Stanford Photonics Research Institute

SPEAKERS

William D. Phillips, National Institute of Standards and Technology, Gaithersburg, MD
Laser Cooling and Trapping: Making the Coldest Stuff in the Universe
David N. Payne, University of Southampton, United Kingdom
How Lasers and Glass Fibers Changed Our World
Edward Moses, Lawrence Livermore National Laboratory, Livermore, CA
National Ignition Facility: Creating Star Power in the Laboratory

Special Session

2010 Forum for Sustainability Science Programs

Thursday, 18 February

1:00PM–6:00PM

Organized by Arnim Wiek, Arizona State University, Tempe; Amy Fuller, AAAS International Office, Washington, DC

As sustainability considerations rise on both the domestic and international agenda, policy makers at all levels of governance increasingly look to scientists and engineers to provide guidance in creating sustainable societies. Universities have responded by developing academic and research programs in Science and Technology for Sustainable Development or "Sustainability Science" that undertake practical, place-based research to provide decision-support for addressing sustainability challenges.

These inherently interdisciplinary, society-focused programs have converged on the AAAS Annual Meeting as the most appropriate meeting place for networking and exchanging ideas. Beginning with the 2007 Annual Meeting in San Francisco, the AAAS Center for Science, Technology, and Sustainability has convened the AAAS Forum for Sustainability Science Programs.

An important prerequisite to development of course content and curriculum development is having a clear understanding of the core competencies expected of program graduates. The 2010 Forum will focus on this with the ultimate goal of a set of commonly agreed-upon competencies. For more information on this invitation-only event, contact Amy Fuller at afuller@aaas.org.

Special AAAS Membership Offer

Do you know colleagues who are not yet members of AAAS?

If they register in advance for the 2010 Annual Meeting in San Diego, they will receive a one-year membership to AAAS along with all member benefits. These include a one-year subscription to the journal *Science* and online access to *Science*, all of its archives, and *Science Express*. International members will receive *Science Digital*.

This offer is good for advance registration only, and expires on 28 January 2010. Only nonmembers qualify.

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Symposium Tracks

Beyond the Classroom

Building Bridges Between Ocean Scientists, Educators, and Students

Organized by Gwen Noda, University of California, Los Angeles; Linda Duguay, University of Southern California, Los Angeles

Civic Scientific Literacy in Developed and Developing Countries

Organized by Jon D. Miller, Michigan State University, East Lansing; Rajesh Shukla, National Council of Applied Economic Research, New Delhi, India

Learning Science in Informal Environments

Organized by Bruce V. Lewenstein, Cornell University, Ithaca, NY

Mind Changes: Can Out-of-School Learning Contribute to Evolution Literacy?

Organized by Martin Weiss, New York Hall of Science, New York City

Reemergence of Science, Technology, and Education as Priorities in the Arab World

Organized by Ashley Dougherty and Cindi Warren Mentz, U.S. Civilian Research Development Foundation, Arlington, VA

Scientific Foundations for Future Physicians

Organized by Jodi Lubetsky and Anthony Mazzaschi, Association of American Medical Colleges, Washington, DC

Strategies for Diaspora To Be Enablers of S&T Capacity-Building in Their Homelands

Organized by Pallavi Phartiyal, AAAS Science and Policy Programs, Washington, DC; Lara Campbell, CUBRC Center for International Science and Technology Advancement, Washington

Tomorrow's Scientists and Engineers

Organized by Jon D. Miller, Michigan State University, East Lansing; Greg Pearson, National Academy of Engineering, Washington, DC

Top-Down or Bottom-Up? Comparing European and U.S. Gender Policies in Science

Organized by Marina Marchetti, European Commission, Directorate General for Research, Brussels, Belgium

Women and Men in the Scientific Work Force: Issues of Networks, Partners, and Ethics

Organized by Julia E. Melkers, Georgia Institute of Technology, Atlanta

Cognitive Function and Development

The Brain on Trial: Neuroscience Evidence in the Courtroom

Organized by Deborah Runkle and Mark S. Frankel, AAAS Science and Policy Programs, Washington, DC

From Gene Discovery to Cell Biology in Psychiatry: An Emerging Case

Organized by Tyrone Cannon, University of California, Los Angeles

Language Learning in Deaf Children: Integrating Research on Speech, Gesture, and Sign

Organized by Jenny Saffran, University of Wisconsin, Madison

Language Processing for Science and Society

Organized by Annie Zaenen, Palo Alto Research Center, CA

The Long Reach of Early Childhood Poverty: Pathways and Impacts

Organized by Greg J. Duncan, University of California, Irvine

Music-Language Interactions in the Brain: From the Brainstem to Broca's Area

Organized by Aniruddh D. Patel, Neurosciences Institute, San Diego, CA

Role of Sleep in Memory from Development to Old Age

Organized by Sara C. Mednick, University of California, San Diego

Stress and the Central Role of the Brain in Health Inequities

Organized by Michael J. Zigmond, University of Pittsburgh, PA; Bruce S. McEwen, Rockefeller University, New York City

Traumatic Brain Injury: The Violent and Silent Epidemic

Organized by Mahlon DeLong and David Wright, Emory University School of Medicine, Atlanta, GA

Unexpected Discoveries on Brain Function and Development from Model Organisms

Organized by S. Lawrence Zipursky, University of California, Los Angeles; Barbara Illman, U.S. Forest Service, Madison, WI

Communicating Science

Communicating Science to the Public: Culture and Social Context in East Asia

Organized by Masataka Watanabe, Japan Science and Technology Agency, Tokyo, Japan; Sook-Kyoung Cho, Korea Foundation for the Advancement of Science and Creativity, Seoul; Sun Mengxin, China Association for Science and Technology, Beijing

Communicating on the State and Local Level: How Can Scientists Support Policy-Makers?

Organized by Peyton West and Erin Heath, AAAS Science and Policy Programs, Washington, DC

Covering Global Climate Change and Adaptation from the Ground Up

Organized by Cristine Russell, Harvard University, Cambridge, MA; Deborah Blum, University of Wisconsin, Madison; Phillip Hilts, MIT's Knight Science Journalism Fellowships, Cambridge, MA

Earthquake Science and Advocacy: Helping Californians Live Along the San Andreas Fault

Organized by Mark L. Benthien, Southern California Earthquake Center, Los Angeles

Eyes on Screen: Communicating Science in the New Information Age

Organized by Sharon Dunwoody, University of Wisconsin, Madison; Lynne Friedmann, Friedmann Communications, Solana Beach, CA

Facing the Uncertain Future of International Science Journalism

Organized by Cristine Russell, Harvard University, Cambridge, MA; James Cornell, International Science Writers Association, Tucson, AZ; Donald Kennedy, Stanford University, CA

Genetics and Ethics: Different Views on the Human Condition

Organized by Walter Doerfler, University of Cologne, Erlangen, Germany; Hans G. Ulrich, Erlangen University, Germany

Plato's Progeny: Academies of Science

Organized by Lynn E. Elfner, Ohio Academy of Science, Columbus; Jay B. Labov, National Research Council, Washington, DC

Science in the Theater

Organized by Vince LiCata, Louisiana State University, Baton Rouge

Science Meets Society: Walking the Talk

Organized by Viviane Willis-Mazzichi and Raffaella Di Iorio, European Commission, Joint Research Center, Brussels, Belgium

Watching the Watchmen and Cheering the Heroes: The Science of Superheroes

Organized by Cortney Riese Sloan and Ann Merchant, National Academies, Washington, DC; Jennifer Ouellette, National Academy of Sciences, Los Angeles, CA

Education in the Classroom

Can Singapore Mathematics Enhance Student Learning in the United States?

Organized by Patsy Wang-Iverson, Gabriella and Paul Rosenbaum Foundation, Stockton, NJ

Demonstrating the Legal Sustainability of Effective STEM Diversity Programs

Organized by Daryl E. Chubin, AAAS Education and Human Resources, Washington, DC

Education Research at Minority-Serving Institutions: What Have We Learned?

Organized by Marilyn J. Suiter, National Science Foundation, Arlington, VA

First-Person Solvers? Learning Mathematics in a Video Game

Organized by Keith Devlin, Stanford University, CA

Role of Community Colleges in Increasing Minority Students in the STEM Pipeline

Organized by Anne Jane MacLachlan, University of California, Berkeley

Science Literacy: How To Train Teachers, Engage Students, and Maximize Learning

Organized by Michael W. Klymkowsky, University of Colorado, Boulder

Scientific Approaches to Teaching Science in K-16 Education

Organized by Robert E. Fay, Westat, Bethesda, MD

TIMSS 2007: Exploring the Dramatic Improvements in Performance in Two States

Organized by Patsy Wang-Iverson, Gabriella and Paul Rosenbaum Foundation, Stockton, NJ

Visualizations in the Mind and in the World: Implications for STEM Education

Organized by Mary Hegarty, University of California, Santa Barbara

Worlds of Wonder: Can Video Games Teach Science?

Organized by Yasmin Kafai, University of Pennsylvania, Philadelphia; Douglas Clark, Vanderbilt University, Nashville, TN

Energy Today and Tomorrow

Advanced Nuclear Energy Concepts for a Safe, Sustainable, Carbon-Free Future

Organized by Tomas Diaz de la Rubia, Lawrence Livermore National Laboratory, Livermore, CA; Robert Rosner, Argonne National Laboratory, Argonne, IL

Biofuels' Uncertain Future: Unraveling the Science and Politics of Indirect Land Use

Organized by Holly K. Gibbs, Stanford University, CA; Richard Plevin, University of California, Berkeley

Combating Global Emissions: The Urgent Need for a New Strategy in the Asia-Pacific Rim

Organized by Elyn M. Murphy and Yong Wang, Pacific Northwest National Laboratory, Richland, WA

Consequences of Changes in Energy Return on Energy Invested

Organized by Carey King, University of Texas, Austin

Gray Is the New Green: How Energy Recycling Curbs Both Global Warming and Power Costs

Organized by Thomas Casten, Recycled Energy Development, Westmont, IL

Nanotechnology: Will Nanomaterials Revolutionize Energy Applications?

Organized by S. Thomas Picraux, Los Alamos National Laboratory, Los Alamos, NM

Smart and Secure Transmission Grids To Realize U.S. and E.U. Renewable Energy Potentials

Organized by Gianluca Fulli and Giovanni De Santi, European Commission, Joint Research Center, Petten, Netherlands

Societal Strategies for Addressing the Climate and Energy Challenge

Organized by Jane C.S. Long, Lawrence Livermore National Laboratory, Livermore, CA

Nuclear Waste Management: From Public Perception to Industrial Reality

Organized by Didier J. Haas, European Commission, Joint Research Center, Brussels, Belgium

Toward Green Mobility: Integrating Electric Drive Vehicles and Smart Grid Technology

Organized by Kathryn Clay, Alliance for Automotive Manufacturers, Washington, DC; Tina Kaarsberg, U.S. Department of Energy, Washington, DC

Urban Design and Energy Demand: Transforming Cities for an Eco-Energy Future

Organized by Nancy Levinson, Arizona State University, Tempe

Global Science and Policy

Bottom-of-the-Economic-Pyramid Technological Solutions: Lessons from Success Stories

Organized by William S. Kisaalita, University of Georgia, Athens

Building International Security Through Lab-to-Lab Exchanges

Organized by Benn Tannenbaum, AAAS Center for Science, Technology, and Security Policy, Washington DC, DC

Information Technologies and Remote Sensing for Understanding Human Rights Violations

Organized by Lars Bromley, AAAS International Office, Washington, DC

Mobilizing East Asian Science and Technology To Address Critical Global Challenges

Organized by Asuka Hoshikoshi and Yuko Nagano, National Institute of Science and Technology Policy, Tokyo, Japan

The Next Big Thing: Keys in the Transformation from Science to Society

Organized by Gerald Hane, Q-Paradigm, Rockville, MD

Oceans Apart? Transatlantic Perspectives on Public Research and Business Innovation

Organized by Eamonn Cahill, Office of the Chief Scientific Adviser, Dublin, Ireland

Privacy in a New Global Context: Trapped Between Culture, Laws, and Technology

Organized by Stephan Lechner, JRC Institute for the Protection and Security of the Citizen, Ispra, Italy; Aidan Gilligan, European

Commission, Joint Research Center,
Brussels, Belgium

Science Academies in Society

Organized by Daniel Schaffer and Tasia Asakawa, Academy of Sciences for the Developing World, Trieste, Italy

What Went Wrong with the Global Economy?

Organized by Rolf Sinclair, Center for Scientific Studies, Valdivia, Chile; J. Doyme Farmer, Santa Fe Institute, NM

When Science Goes Global, Can Everybody Win?

Organized by Sieglinde Gruber, Alessandro Damiani, and Mary Kavanagh, European Commission, Directorate General for Research, Brussels, Belgium

Working Together for the Public: Challenges for Verification of Nuclear Activities

Organized by Aidan Gilligan, European Commission, Joint Research Center (JRC), Brussels, Belgium

Health, Medicine, and the Environment

Applying Biogenomics to Ecology: From the Molecular to the Ecosystem Level

Organized by Teresa Lettieri, JRC Institute for Environment and Sustainability, Ispra, Italy

A California Roadmap for Identifying Chemicals that Affect Breast Cancer Risk

Organized by Sarah Janssen, Natural Resources Defense Council (NRDC), San Francisco, CA; Gabriela Chavarria, NRDC, Washington, DC

Consequences of Endocrine Disrupting Agents in the Laboratory and Home

Organized by John G. Vandenberg, North Carolina State University, Raleigh; A. Wallace Hayes, Harvard School of Public Health, Andover, MA

False Discoveries and Statistics: Implications for Health and the Environment

Organized by Ron Brookmeyer, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; Robert E. Fay, Westat, Bethesda, MD

The Impact of Genomics

Organized by Stephen G. Oliver, University of Cambridge, United Kingdom

Innate Immunity: Theme and Variations

Organized by David H. Raulet, University of California, Berkeley; Christine A. Biron, Brown University, Providence, RI; Sondra Schlesinger, Washington University School of Medicine, St. Louis, MO

Moving Across Scales: Mathematics for Investigating Biological Hierarchies

Organized by Louis J. Gross, University of Tennessee, Knoxville

Mutators Versus Antimutators in Evolution and Medicine

Organized by Robert C. von Borstel, University of Alberta, Edmonton, Canada

One Health: Attaining Optimal Health for People, Animals, and the Environment

Organized by Barbara Hyde, American Society for Microbiology, Washington, DC

Protecting the Consumer: Can “Omics” Keep the Promise?

Organized by Aidan Gilligan, European Commission, Joint Research Center, Brussels, Belgium

Rethinking the Science, Biology, and Importance of Stem Cells in Regenerative Medicine

Organized by Irving Weissman, Stanford University School of Medicine, Palo Alto, CA; Sondra Schlesinger, Washington University School of Medicine, St. Louis, MO; Carol Newlon, University of Medicine and Dentistry of New Jersey, Newark

Science of the Small: Nano-Bio-Technology Under the Biological Microscope

Organized by Barbara Illman, U.S. Forest Service, Madison, WI; Vicki Colvin, Rice University, Houston, TX

Physical Sciences Frontiers

50 Years of Exobiology and Astrobiology: Past, Present, and Future Life in the Universe

Organized by Linda Billings, George Washington University, Washington, DC; Jeffrey Bada, University of California, San Diego

Are Neutrinos the Reason We Exist?

Organized by Kurt Riesselmann, Fermi National Accelerator Laboratory, Batavia, IL

The Arrow of Time

Organized by Sean M. Carroll, California Institute of Technology, Pasadena

Astrobiology and the Future: Science, Ethics, and Societal Issues on Earth and Beyond

Organized by Margaret Race, SETI Institute, Mountain View, CA

Doomsday Versus Discovery

Organized by Renilde Vanden Broeck, CERN, Geneva, Switzerland; Katie Yurkewicz, Fermi National Accelerator Laboratory, Batavia, IL

How Computational Science Is Tackling the Grand Challenges Facing Science and Society

Organized by Edward Seidel, Carmen Whitson, and José Muñoz, National Science Foundation, Arlington, VA

Managing the Exaflood: Enhancing the Value of Networked Data for Science and Society

Organized by Bonnie C. Carroll, Information International Associates Inc., Oak Ridge, TN; Paul F. Uhler, National Research Council, Washington, DC

Particles and People: How Basic Physics Benefits Society

Organized by Elizabeth Clements and Katie Yurkewicz, Fermi National Accelerator Laboratory, Batavia, IL

Physics and Art: A Gateway to the Sciences

Organized by Christopher M. Smith, Center for Theoretical Biological Physics, La Jolla, CA

Real Numbers: Mathematical Technologies for Counterterrorism and Border Security

Organized by Jonathan D. Farley, Johannes Kepler University Linz, Austria; Tony Harkin, Rochester Institute of Technology, NY; Anice Anderson, Rose-Hulman Institute of Technology, Terre Haute, IN

SETI Turns 50

Organized by Jill C. Tarter, SETI Institute, Mountain View, CA

Traffic, Crowds, and Society

Organized by Nicola Bellomo, Polytechnic University of Turin, Torino, Italy; Andrea Bertozzi, University of California, Los Angeles

What's Next for the Net? The Internet of Things and Ubiquitous Computing

Organized by Michael R. Nelson, Georgetown University, Washington, DC

Protecting Marine Resources

Adam Smith Meets Jacques Cousteau: Using Economics To Protect Marine Resources

Organized by Benjamin Halpern, University of California, Santa Barbara; Anne Guerry, National Oceanic and Atmospheric Administration (NOAA) Northwest Fisheries Science Center, Seattle, WA

Confronting Ocean Acidification: Options for Management and Policy

Organized by Susan Park, National Research Council, Washington, DC; Victoria J. Fabry, California State University, San Marcos

Denial, Detente, and Decisions: Fisheries Science at the Crossroads

Organized by Alison Rieser, University of Hawaii at Manoa, Honolulu, HI; John Lynham, University of Hawaii at Manoa, Honolulu, HI

Designing the Future Ocean: Baseline Data Needs for Marine Spatial Planning

Organized by Alison Chase and Lisa Suatoni, Natural Resources Defense Council (NRDC), New York City; Gabriela Chavarria, NRDC, Washington, DC

Ensuring Marine Policy Is Responsive to Social Dynamics and Management Experience

Organized by Patrick Christie, University of Washington, Seattle; Richard Pollnac, University of Rhode Island, Kingston

Land-Ocean Linkages and Dynamics of High-Productivity Ecosystems in the Sea of Cortes

Organized by Exequiel Ezcurra, University of California, Riverside

Limits to Sustainability of Coral Reef Fisheries

Organized by Ayana Elizabeth Johnson, University of California, San Diego

Management and Governance in a Melting Marine Arctic: Challenges and Opportunities

Organized by Lisa Speer, NRDC, New York City; Gabriela Chavarria, NRDC, Washington, DC

Marine Reserves in a Changing World: Connecting Research with Human Needs

Organized by Steven Gaines, University of California, Santa Barbara; Kirsten Grorud-Colvert, Oregon State University, Corvallis; Sarah Lester, University of California, Santa Barbara

One Fish, Two Fish, Red Fish, New Fish: Society Needs Marine Biodiversity Research

Organized by Heather Mannix, Consortium for Ocean Leadership, Washington, DC

Unraveling the Mysteries of the Deep: Effects of Human Activities on Marine Megafauna

Organized by Rebecca Lewison, San Diego State University, CA

Will Coral Reefs Disappear? Separating Fact from Conjecture

Organized by Joanie Kleypas, National Center for Atmospheric Research, Boulder, CO; Kimberley Yates, U.S. Geological Survey, St. Petersburg, FL

Public Health and Wellness

Children of Assisted Reproductive Technologies: Their Health and New Genetic Issues

Organized by Marvin L. Meistrich, University of Texas M.D. Anderson Cancer Center, Houston

Decoding the Secret Pathologies of Dolphins: Significance for Human and Ocean Health

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Massachusetts Institute of Technology, Cambridge

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Up in Flames: Fire in a Changing Environment

Organized by Susan G. Conard, U.S. Forest Service (retired), Silver Spring, MD



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From the Journal *Science*

POSITIONS OPEN



CELL AND SYSTEMS BIOLOGY University of Toronto

The Department of Cell & Systems Biology at the University of Toronto invites applications for a tenure-track faculty position to be appointed at the level of **ASSISTANT** or **ASSOCIATE PROFESSOR** beginning July 1, 2010.

We are interested in outstanding candidates that complement existing strengths in the Department in the areas of genomics, cell and developmental biology, and neurophysiology using high-throughput approaches or gene/protein network analyses with genomic, proteomic, or advanced cell biological and imaging tools. We particularly encourage applications from candidates who are addressing fundamental questions in plant and microbial biology, but will consider all outstanding candidates within the framework of our Department. For more information about the Department of Cell & Systems Biology, please visit our home page at website: <http://www.csb.utoronto.ca/>.

Candidates should have at least two years of research experience beyond their doctoral degree. In addition to pursuing a vigorous, internationally recognized research program, the successful candidate will demonstrate potential for excellence in undergraduate and graduate teaching in the molecular life sciences. The successful candidate would also be expected to network with researchers across the University to take advantage of the extensive resources in cell and systems biology at the University of Toronto and its affiliated institutions. Salary will be commensurate with qualifications and experience.

Qualified candidates are encouraged to submit their applications online at website: <http://www.jobs.utoronto.ca/faculty.htm>. Applicants should submit a single PDF file (less than two megabytes) that includes a cover letter, their curriculum vitae, and statements of research and teaching interests. Applicants should also arrange for at least three confidential letters of recommendation to be sent directly to: Prof. Ulrich Tepass, Chair, Department of Cell & Systems Biology, University of Toronto, 25 Harbord Street, Toronto, Ontario M5S 3G5, Canada. Or to e-mail: search@csb.utoronto.ca by January 15, 2010.

The University of Toronto is strongly committed to diversity within its community and especially welcomes applications from visible minority group members, women, Aboriginal persons, persons with disabilities, members of sexual minority groups, and others who may contribute to the further diversification of ideas. All qualified candidates are encouraged to apply; however, Canadians and permanent residents will be given priority.

The Department of Neuroscience at The Ohio State University plans to hire several junior, tenure-track **NEUROSCIENTISTS** beginning this academic year. The Neurosciences Signature Program, which also comprises Neurology and Neurological Surgery, has been targeted for investment at the Ohio State University Medical Center. Although we are interested in applicants who demonstrate an exceptional record of research accomplishment in basic neuroscience, we are particularly interested in individuals whose research interests build on current or emerging strengths in spinal cord and brain injury, neurophysiology, and mechanisms of neural degeneration/regeneration and who provide translational links to our clinical colleagues. State-of-the-art research facilities are available. Applicants should submit curriculum vitae, a statement of research interests, and three letters of reference to: Randy J. Nelson, Department of Neuroscience, Ohio State University, Columbus, OH 43210. Application materials may be submitted electronically (preferably in PDF format) to e-mail: cheryl.yoder@osumc.edu. Application deadline: December 15, 2009. *The Ohio State University has a strong commitment to diversity and actively encourages applications from individuals of underrepresented groups. The Ohio State University is an Equal Opportunity/Affirmative Action Employer.*

POSITIONS OPEN

FACULTY POSITIONS COMMUNITY ECOLOGY

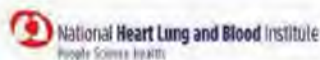
The Ecology Department at Montana State University seeks two community ecologists at the level of **ASSISTANT PROFESSOR**: one terrestrial animal community ecologist and one plant community ecologist. We seek individuals with interests in fundamental ecological questions and the use of ecological theory to solve important applied problems and who have strong interests in collaboration. The Department of Ecology is committed to quality undergraduate and graduate education, and the successful candidate is expected to participate actively in the Department curriculum, including courses at both the undergraduate and graduate levels. Successful candidates will be expected to maintain an excellent program of research and publication with support from national competitive-grant programs, and to provide graduate student support and mentoring. Full position descriptions and application information are available at websites: <http://www.montana.edu/cgi-bin/msuinfo/fpview/f/1019-2> and <http://www.montana.edu/cgi-bin/msuinfo/fpview/f/1024-2> (plant ecologist and animal ecologist, respectively). Screening of applications will begin January 15, 2010, and will continue until a suitable candidate is found. Electronic submittal of applications is highly preferred.

Montana State University is an institution that is committed to cultural diversity and encourages applications from members of underrepresented groups to include women and minority candidates.

TENURE TRACK POSITION in Biochemistry and Molecular/Cellular Biology

The Department of Biology at the University of Louisville, website: <http://louisville.edu/biology>, invites applications for a tenure-track position at the **ASSISTANT** or **ASSOCIATE PROFESSOR** level to begin fall 2010. This position is open to individuals using any eukaryotic model system. Research expertise and interests may include, but are not limited to: metabolism, metabolic regulation, mechanisms of host defense, hormonal regulation and development. Individuals with research programs that target insect, fungal, or plant systems or with research programs with a focus in developmental biology (any system) are strongly encouraged to apply. Postdoctoral experience is required. The successful candidate is expected to contribute to the Department's teaching mission within both the undergraduate and graduate programs and to maintain an excellent record of research productivity and external funding. Primary teaching duties will include contributions to the core undergraduate curriculum in cellular and molecular biology and teaching graduate-level material in the applicant's area of specialization. Applicants must apply online at website: <http://www.louisville.edu/jobs>, Job #24657 and attach only curriculum vitae. Additionally, applicants should submit curriculum vitae, statements of research and teaching interests, representative reprints, and contact information for three references to: **BMCB Search Committee, Department of Biology, University of Louisville, Louisville, KY 40292**. Review of applications will begin on December 1, 2009, and will continue until the position is filled. *The Department of Biology is committed to building a culturally diverse faculty. The University of Louisville is an Affirmative Action, Equal Opportunity, Americans with Disabilities Employer, committed to diversity and in that spirit, seeks applications from a broad variety of candidates.*

Engineering and Public Policy at Carnegie Mellon seeks doctoral students with technical backgrounds to address policy issues such as: electric power, energy and environment, advanced vehicles, nanotechnology, climate change, cybersecurity, privacy, information technology policy, risk analysis and regulation, management of innovation and R&D. Opportunities for dual degrees with Portugal available. See website: <http://www.epp.cmu.edu>. Victoria Finney, Engineering and Public Policy, Carnegie Mellon, Pittsburgh, PA 15213 U.S.A.



Deputy Scientific Director

The Division of Intramural Research (DIR) of the National Heart, Lung and Blood Institute (NHLBI) is seeking an exceptional candidate for the position of Deputy Scientific Director to provide leadership and support as an active partner with the Scientific Director in leading a large research program. The research program is wide in scope including both basic and clinical scientific research programs in such areas as heart and vascular disease, blood diseases, pulmonary, cardiology, hematology, cell biology, genetics, immunology, biophysics, and biochemistry. The existing faculty is an outstanding group of internationally recognized biomedical researchers covering a wide range of basic and clinical research topics (<http://dir.nhlbi.nih.gov/>).

This position offers a unique and exciting opportunity for the right individual to share responsibility in providing visionary leadership to an organization dedicated to uncovering new knowledge and technologies, both basic and clinical. A candidate is sought who has a commitment to scientific excellence to help identify emerging areas of opportunity for collaboration and to work with members of the research community to implement strategies for successful research outcomes. The incumbent will serve as the liaison between the DIR and the NHLBI Board of Scientific Counselors (BSC) with full oversight responsibilities for the entire BSC process. He/she will serve as a partner to establish relationships with regional hospitals to expand research opportunities and to impact clinical care. The incumbent will also build trans-NIH scientific and clinical collaborations and participate in trans-NIH initiatives. The candidate is expected to perform the specific duties listed above in addition to co-directing the intramural activities of the DIR with the Scientific Director.

Applicants must have an M.D., Ph.D., or both as well as senior-level research experience or knowledge of research programs in one or more scientific areas, related to the above mentioned DIR areas of interest. The candidate shall have administrative experience running a complex research program or institution. The candidate should be a strong communicator with the ability to work collaboratively to solve problems and to make informed decisions.

The successful candidate will be offered a competitive salary commensurate with experience and qualifications with a full benefits package (retirement, health & life insurance, leave, etc.). Appointees may be US citizens, resident aliens, or non-resident aliens with or eligible to obtain a valid employment authorized visa. Complete applications must be received by December 18, 2009. Review of applications is expected to begin in late December, but applications will be accepted until the position is filled. Please submit a curriculum vitae and three letters of reference in .pdf or Microsoft word format only (no paper applications will be accepted) to: **Robert S. Balaban, Ph.D., Scientific Director, NHLBI, c/o Tara Terndrup, nhlbideputysearch@mail.nih.gov**



National Institutes of Health National Institute of Arthritis and Musculoskeletal and Skin Diseases, Intramural Research Program

Tenured/Tenure-Track Investigator(s)

The Intramural Research Program of the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) of the National Institutes of Health (NIH) in the Department of Health and Human Services (DHHS) is recruiting outstanding tenure-track and/or senior (tenured) scientists (M.D., Ph.D., or M.D./Ph.D) active in any of the following areas relevant to musculoskeletal biology or diseases:

- > Basic, Translational and Clinical Research in Orthopaedics, Bone, Cartilage, or Muscle
- > Nanotechnology related to Bone, Cartilage, Tendon/Ligaments, or Muscle
- > Biology of inducible pluripotent stem (iPS) and mesenchymal stem cells for the study of human disorders
- > Biological/Tissue Engineering
- > Regenerative Medicine

Emphasis will be placed on the applicants' demonstrated track record of high-quality research and the originality and promise of their future plans. Successful applicants will be expected to develop energetic, creative, independent research programs within an existing highly interactive scientific environment. The ideal candidate would benefit from pre-existing expertise within NIAMS.

This position(s) is located on the NIH campus in Bethesda, Maryland, a suburb of Washington, D.C. NIAMS and the NIH offer tremendous depth and breadth of intellectual and technological resources, as well as opportunities for collaboration with investigators both within and outside of the NIH. NIAMS is also a major user of the NIH Clinical Research Center, a state-of-the-art research hospital on the campus of the NIH in Bethesda, Maryland. The research environment is highly conducive to advancing basic and translational research and highly collaborative, encouraging multidisciplinary and interdisciplinary team science.

The mission of NIAMS is to support research into the causes, treatment, and prevention of arthritis and musculoskeletal and skin diseases, the training of basic and clinical scientists to carry out this research, and the dissemination of information on research progress in these diseases.

Applicants should submit a cover letter that includes a short research interest statement (two page maximum), a curriculum vitae and complete bibliography, along with complete contact information of three referees. Applications should be submitted by **February 1, 2010**.

Applications should be submitted to: **Mrs. Wanda White – RE: Musculoskeletal Initiative, Building 31 Room 4C-12, 9000 Rockville Pike, Bethesda MD 20892, Email: whitewan@mail.nih.gov**

NIAID Transition Program in Clinical Research

The National Institute of Allergy and Infectious Diseases (NIAID) Transition Program in Clinical Research provides an opportunity for physicians to gain clinical and translational research experience in the NIAID Division of Intramural Research (DIR). The program aims to increase the pool of well-trained clinical investigators who are competitive for clinical tenure-track positions.

Up to three candidates per year will be selected for three- to five-year appointments as assistant clinical investigators. Applicants must have an M.D. or M.D./Ph.D., be board eligible or board certified in a subspecialty (or equivalent), and qualify for credentialing by the NIH Clinical Center. Applicants should identify a DIR lab chief who will agree to host their research. Information about DIR labs and contact information for lab chiefs is available at www.niaid.nih.gov/about/organization/dir/.

Applications will be evaluated by a search committee composed of NIAID DIR principal investigators with clinical and basic research interests. Competitive candidates will be asked to present their research accomplishments and plans to the search committee.

Participants will receive independent resources and staff and will be mentored by an NIAID senior clinical investigator.

Interested candidates may contact DIR Deputy Director Dr. Karyl Barron at 301-402-2208 or kbarron@nih.gov for additional information or assistance in identifying an appropriate host lab.

To apply for the program, e-mail curriculum vitae/bibliography, a research program proposal (no more than two pages), and a letter of support from the accepting NIAID lab chief by **November 30, 2009** to Ms. Yushekia Hill at

NIAID.DIR.Search@niaid.nih.gov.

In addition, send three letters of recommendation to Chair, Transition Program in Clinical Research Search Committee, c/o Ms. Yushekia Hill by e-mail at NIAID.DIR.Search@niaid.nih.gov or by post at 10 Center Drive, MSC 1356, Building 10, Room 4A-22, Bethesda, MD 20892-1356. E-mail is preferred. Please note search #029 when sending materials.

NIAID

National Institute of Allergy and Infectious Diseases

Further information about working at NIAID is available on the Web at www.niaid.nih.gov/careers/stp.



U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
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Tenure-Track/Tenured Investigator Laboratory of Immunology

The Laboratory of Immunology (LI), Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIH) invites applications for a tenure-track/tenured investigator position in immunology. Applicants should have a Ph.D., M.D., or equivalent degree; an outstanding record of postdoctoral accomplishment; and an interest in any area of biomedical research related to immunology.

Specifically, we seek a highly creative individual who will establish an independent, world-class research program that takes full advantage of the special opportunities afforded by the stable, long-term funding of the intramural research program at NIH. She or he should be interested in developing and applying novel approaches to the study of problems of major biological and/or medical importance, which could include a significant clinical or translational effort in addition to bench research. In the former case, the successful candidate would have access to the NIH Clinical Center, a state-of-the-art research hospital on the NIH campus in Bethesda, MD, and ample opportunity to participate in the activities of the Trans-NIH Center for Human Immunology.

Generous ongoing support for salary, technical personnel, postdoctoral fellows, equipment, and research supplies will be provided. Available core or collaborative facilities include flow cytometry, advanced optical imaging, microarray generation and analysis, high throughput sequencing, computational biology, production of transgenic and gene-manipulated mice, biosafety level (BSL)-3 facilities, chemical genomics, and support for projects involving RNAi screening. The successful applicant will also have access to Trans-NIH initiatives involving technology development, translational investigation, and multidisciplinary science. In addition to an outstanding international postdoctoral community, a superior pool of graduate and undergraduate students is available to the successful applicant.

LI has a distinguished history of accomplishment in immunology. We strongly encourage application by outstanding investigators who can continue and enhance this record of achievement. Current LI investigators are Ronald Germain, Michael Lenardo, David Margulies, Stefan Muljo, William Paul, Ethan Shevach, and Tsan Xiao.

To apply, e-mail curriculum vitae, bibliography, and outline of a proposed research program (no more than two pages) to Ms. Yushekia Hill at NIAID.DIR.Search@niaid.nih.gov. In addition, three letters of reference must be sent directly from the referee to Drs. Giorgio Trinchieri and Dan Kastner, Co-Chairs, NIAID Search Committee, c/o Ms. Yushekia Hill, at NIAID.DIR.Search@niaid.nih.gov or 10 Center Drive, MSC 1356, Building 10, Room 4A22, Bethesda, MD 20892-1356. E-mail is preferred. Applications will be reviewed starting **11/16/09** and will be accepted until the position is filled. Please refer to ad #028 on all communications. For further information about this position, contact Dr. William Paul at 301-496-5046 or wpaul@niaid.nih.gov.

A full package of benefits (including retirement and health, life, and long-term care insurance) is available. Women and minorities are especially encouraged to apply. U.S. citizenship is not required.

NIAID

National Institute of Allergy and Infectious Diseases

To learn more about NIAID and how you can work in this exciting research organization, please visit us on the web at www.niaid.nih.gov/careers/sti.



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Edgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich

Assistant Professor (Tenure Track) of Neurogenetics

The Department of Biology (www.biol.ethz.ch) at ETH Zurich invites applications for an Assistant Professor (Tenure Track) in the area of Neurogenetics.

The candidate is expected to build a strong and independent research program in neurobiology aimed at studying the relationship between the genome and complex traits such as behaviour, and the underlying biological mechanisms, both in health and disease. An orientation towards the field of neuroepigenetics at an organism level will be highly considered. A solid background in genetics/epigenetics, molecular biology, physiology, behavioral and cognitive neuroscience, and in the design and use of animal and cellular models is highly desired. The successful applicant is expected to employ molecular methods including genetic engineering in rodents, virus-mediated gene manipulations, and RNA interference, in combination with behavioral, electrophysiological, biochemical, and/or proteomics approaches to modulate molecular pathways and examine the impact in vivo and in vitro. A tight integration into the major strategic research areas of the Department of Biology, including Movement Sciences and Energy Homeostasis, Metabolism and Aging, and Tissue Remodelling, Inflammation and Repair is foreseen.

The future Assistant Professor will be a member of the Department of Biology at ETH Zurich. Together with Life Science Zurich, the Department of Biology offers outstanding scientific opportunities to contribute to interdisciplinary research programs and establish close interactions with the local community through the Center for Neuroscience Zurich, the National Center for Competence in Research «Neural Plasticity and Repair», SystemsX.ch, and the Functional Genomics Center Zurich. An involvement in the Biology and Neuroscience teaching program is expected. She or he will be expected to teach undergraduate level courses (German or English) and graduate level courses (English).

Assistant professorships have been established to promote the career of young and talented scientists. The initial appointment is for four years with the possibility of renewal for an additional two-year period and promotion to a permanent position.

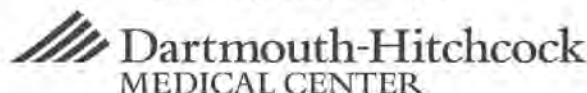
Please submit your application including a curriculum vitae, list of publications and detailed research plan to the President of ETH Zurich, Prof. Dr. Ralph Eichler, ETH Zurich, Raemistrasse 101, 8092 Zurich, Switzerland, no later than January 31, 2010. With a view towards increasing the proportion of women in the faculty, ETH Zurich specifically encourages women to apply.

INFECTIOUS DISEASE & INTERNATIONAL HEALTH SECTION CHIEF

Dartmouth Medical School and Dartmouth-Hitchcock Medical Center (DMS/DHMC) invite applications for the position of Chief, Section of Infectious Disease & International Health within the Department of Medicine. This individual will lead the clinical, educational, and research missions of a comprehensive academic program. DHMC is a 400-bed tertiary care hospital in New Hampshire, with 500 medical and graduate students, and, along with its affiliate VA Medical Center, is a major teaching hospital for DMS. The successful candidate will be BC in Internal Medicine and Infectious Disease with active clinical responsibilities and have an outstanding record of scholarly achievement and original research and sustained extramural research funding. The candidate should also possess excellent interpersonal and mentoring skills as well as administrative acumen and experience. Applicants must qualify for a senior academic appointment as Associate or full Professor of Medicine at DMS. Additional research/academic opportunities include participation in: programs in international infectious diseases, such as the potential for involvement in the DarDar Programs in Tanzania on HIV and M. tuberculosis, and the Dartmouth-Boston University Fogarty AIDS International Training and Research Program. Other opportunities include extensive interactions with the faculty of Microbiology & Immunology, including a secondary academic appointment and collaborative participation in the research programs of faculty members; the NIH-funded Center of Biomedical Research Excellence in Molecular, Cellular, and Translational Immunological Research; The Dartmouth Institute; and Dartmouth College's Dickey Center of International Understanding. Additionally, the recent arrival of Dr. Jim Yong Kim, M.D., Ph.D. and leader of international health care initiatives, as the 17th President of Dartmouth College, provides an extraordinary opportunity for the new Chief of IDIH to interactively address health and infectious disease issues of global importance.

Candidates should submit a curriculum vitae along with a letter of application and the names of three references preferably using email to:

Ronald Taylor, Ph.D., Chair, Search Committee
c/o Laurel Denison, Dept. of Medicine
Dartmouth-Hitchcock Medical Center
One Medical Center Drive, Lebanon, NH 03756
Ronald.K.Taylor@Dartmouth.edu



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COLUMBIA UNIVERSITY
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Neuroscience Faculty Recruitment

The Department of Neuroscience at Columbia University plans to recruit new faculty in two broad areas of neuroscience: (1) the analysis of motor and cognitive processes in awake, nonhuman primates, (2) the use of molecular and cellular approaches to study neural circuitry in genetically tractable model systems. We encourage applications at all levels, from Assistant to Full Professor.

Columbia University has an exceptionally strong and broad program in the neurosciences and aims to enhance interactions between basic and clinical research, and to link the neurosciences with other scientific disciplines within the University. New faculty will be affiliated with the Department of Neuroscience and with the Doctoral Program in Neurobiology and Behavior. There are many opportunities for interaction with other scientific departments and programs at the Medical Center and Morningside Heights campuses.

Applications must be received by November 30, 2009, and should be submitted online at:

<https://academicjobs.columbia.edu/applicants/Central?quickFind=52268>

Columbia University takes affirmative action to ensure equal employment opportunity.

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Year-long fellowships are available in the U.S. Congress and federal agencies. Applicants must hold a PhD or equivalent doctoral-level degree in any behavioral/ social, biological, computational/ mathematical, earth, medical/health, or physical science, or any engineering discipline. Individuals with a master's degree in engineering and three years of post-degree professional experience also may apply. Federal employees are not eligible and U.S. citizenship is required.

Apply.

The application deadline for the 2010-2011 AAAS Fellowships is 15 December 2009. Fellowships are awarded in the spring and begin in September. Stipends range from \$73,000 to \$95,000.

Note: Additional fellowships are available through approximately 30 scientific society partners. Individuals are encouraged to apply with AAAS as well as with any scientific societies for which they qualify.

Full details at:
fellowships.aaas.org



*Enhancing Public Policy,
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Jose Fernandez, PhD
Biochemistry, Purdue
University

2006–07 AAAS Fellow at
the Department of Health &
Human Services, Office of
Public Health & Emergency
Preparedness

Now a senior science advisor
at the U.S. Department of
Health & Human Services,
Office of the Assistant
Secretary for Preparedness
and Response, International
Health Regulations Program

Assistant, Associate or Senior Scientist

The Geology and Geophysics Department invites applications for two full-time tenure-track positions at the Assistant, Associate or Senior Scientist level. These positions are eligible for benefits.

The first position is in the area of metamorphic petrology (fluid-rock interaction) to pursue new research directions in fluid-rock interactions and chemical fluxes between the crust, mantle and oceans, dehydration/serpentinization processes, kinetic and thermodynamic modeling of metamorphic processes and elucidating the linkages between fluid flow, alteration deformation and the chemical fluxes across subduction zones. The candidate would bridge research between geochemists and geophysicists studying the global geochemical cycle from midocean ridges to subduction zones, and the geochemical evolution of the Earth.

The second position is in the area of isotope geochemistry. The successful candidate would apply state-of-the-art technologies of isotope geochemistry to a range of problems, including timescales of geologic processes, global geochemical cycles, mantle dynamics and evolution, biogeochemical cycles, and geochemical kinetics.

Successful candidates are expected to develop and maintain their own independent externally funded research program. Opportunities exist for teaching and advising graduate students through the MIT-WHOI Joint Program in Oceanography/Applied Ocean science and Engineering, as well as for collaborating with scientists and engineers elsewhere in the institution; the Departments of Marine Chemistry and Geochemistry, Biology, Physical Oceanography, Applied Ocean Physics and Engineering, and the Marine Policy Center.

A Ph.D. is required at the time of the appointment as well as a demonstrated record of excellence in research. The level of appointment will depend on the candidate's background and experience. Women and minority applicants are particularly encouraged to apply.

Target for applications, which should include a CV, a research statement and a list of 4 references who could submit letters of recommendation is November 30th, 2009.

Please visit <http://jobs.whoi.edu> for a detailed job description and to apply online today!

WHOI is an Affirmative Action/Equal Opportunity Employer M/F/D/V.
Applications are reviewed confidentially.



Woods Hole Oceanographic Institution



IN 2010
CNRS IS RECRUITING

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- ENVIRONMENT AND SUSTAINABLE DEVELOPMENT
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CNRS encourages junior and senior scientists from around the world to apply for its tenured researcher positions.

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Application forms and further information will be available online at www.cnrs.fr in December 2009



Assistant or Associate Professor MODELING NEUROBIOLOGICAL SYSTEMS/COMPUTATIONAL NEUROSCIENCE

The Division of Biological Sciences (www.biology.missouri.edu) at the University of Missouri-Columbia invites applications for a tenured or tenure-track position in modeling neurobiological systems / computational neuroscience. **We are particularly interested in candidates who develop, expand and evaluate modeling tools to study neurobiological problems.** This is the second in a series of hires designed to increase computational/modeling approaches in our department. The successful candidate will join a diverse group of biologists with interests in neurobiology and behavior, cell and developmental biology, and ecology and evolutionary biology. There are strong possibilities for joint appointments with either Biological Engineering or Electrical/Computer Engineering if the candidate and department(s) are interested.

We offer a highly competitive salary and start-up package, an active doctoral program with institutional and federal support for students, and a highly interactive faculty.

Send application by e-mail to: neuromod@missouri.edu. Attach a single Adobe Acrobat PDF or Microsoft Word document that includes your vita and statements of research and teaching interests. Have three letters of reference mailed to: **John David, Chair, Division of Biological Sciences, 105 Tucker Hall, University of Missouri, Columbia, MO 65211-7400.** Review of applications will begin **December 15, 2009.** To request ADA accommodation contact **Johnette Blair** at 573-882-6650 or BlairJo@missouri.edu.

*MU is an Equal Opportunity-Affirmative Action Employer.
We are committed to ethnic, racial, and gender diversity in our faculty and strongly encourage applications from women and members of groups underrepresented in science.*



Group Leader Position (Population Genetics, Genomics and Evolution)

Gregor Mendel Institute, Vienna, Austria

The **Gregor Mendel Institute of Molecular Plant Biology (GMI)**, founded by the Austrian Academy of Sciences in 2000, is a leading institute in the field of plant molecular biology located at the prestigious Vienna Biocenter Campus. Current research topics include population genetics, epigenetics, chromosome biology, developmental biology and plant stress signal transduction. For more information, see website: www.gmi.oeaw.ac.at

The research topic of the new Group Leader should be in the broad field of plant genomics, systems biology, or population genetics, preferably with a connection to evolution. Side projects involving organisms other than plants are welcome.

The GMI offers internationally competitive packages, including substantial institutional funding and access to state-of-the-art core facilities. The initial contract will be for 5 years (extension is subject to review).

Please send your application, including a curriculum vitae, a description of your proposed research, and contact details for three referees to **Christiane Haffner** (christiane.haffner@gmi.oeaw.ac.at). Review of applications will begin on 1 December 2009. Informal inquiries can be directed to **Dr. Magnus Nordborg** (magnus.nordborg@gmi.oeaw.ac.at).

FELLOWSHIPS

Science Scholarships and Fellowships

UNCF/MERCK SCIENCE INITIATIVE

The UNCF/Merck Science Initiative is an innovative approach that creates opportunities in the biological and chemical sciences for African American students throughout the country.



UNDERGRADUATE Science Research Scholarship Awards

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- Mentoring and networking opportunities
- Eligibility: College juniors, science majors, 3.3 GPA

GRADUATE Science Research Dissertation Fellowships

- Fellowships up to \$52,000
- Mentoring and networking opportunities
- Eligibility: Ph.D. or equivalent degree candidates engaged in dissertation research in biological or chemical research fields

POSTDOCTORAL Science Research Fellowships

- Fellowships up to \$85,000
- Mentoring and networking opportunities
- Eligibility: Ph.D. or equivalent degree recipients in biological or chemical research fields

APPLY ON-LINE

www.uncf.org/merck
Submit by December 1, 2009

T 703 205 3400
F 703 205 3550
E uncfmerck@uncf.org



GENERAL ELIGIBILITY REQUIREMENTS:
Must be African American and a U.S. citizen or a permanent resident



BIG DREAMS. BOLD FUTURE.

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The University of South Florida invites applications for postdoctoral research scientists at the College of Marine Science, a rapidly expanding group spanning all of the major marine science disciplines. These 2 year appointments have competitive salary packages with opportunities to conduct high-quality postdoctoral research in association with selected ranked faculty members. For disability accommodations, please call (727) 553-3942.

VISITING RESEARCH ASSOCIATE/ ENDOWED POSTDOCTORAL

We are seeking an outstanding scientist with a recent doctorate in marine science, oceanography, or a closely related field. The successful candidate should demonstrate excellence in scholarly productivity and academic achievement, and have an expertise that complements a specialty within the College of Marine Science. Successful candidates must meet the completion of the Ph.D. requirements no later than end of Spring 2010 semester at their institution.

Interested candidates should apply online at <https://employment.usf.edu>. Under the Florida Sunshine Law, applications and search committee meetings are open to the public. Please submit a statement of research interests specifying the research contributions you could provide to the College; include name(s) of potential collaborating College of Marine Science faculty, a cover letter, curriculum vitae and contact information for three referees, no later than December 1, 2009. For information regarding this available position (12563), please contact **Dr. Albert C. Hine, Chair, Visiting Research Associate Search Committee, 727-553-1161** hine@marine.usf.edu. Please also visit www.marine.usf.edu.

POSTDOCTORAL FELLOW/ORGANIC & ISOTOPE GEOCHEMISTRY

We are searching for a postdoctoral fellow/research associate to work on a NSF-P2C2 grant funded program to reconstruct late Quaternary paleohydrology and paleoclimate of the Amazon basin. To conduct this research, new sediment cores will be collected from offshore Brazil in 2010. The successful candidate will utilize the abundance of lipid biomarkers (higher plants and soil-derived bacteria) and their isotopic signatures ($\delta^{13}C$ and δD) to evaluate changing continental hydrologic conditions (river discharge, basin hydrology (arid/wet), vegetation changes, continental mean air temperature and soil pH). Together with collaborators at Duke University who will be focusing on foraminifera records of ocean climate variability (SST, E/P, SSS), linkages between ocean climate regimes and the response of the continental hydrologic conditions will be explored.

Applicants must have a strong background in organic geochemistry and/or biogeochemistry and have a PhD with emphasis on geochemistry, paleoenvironment and/or paleoclimate, laboratory experience with methods for lipid extraction, analytical experience with GC-MS/GC-FID and LC/MS for compound, identification and with GC-IRMS coupling for compound-specific isotopic analyses is highly desirable. Applications will be reviewed as received. Expected starting date is February 2010. For the appropriate candidate we would delay the start time but strongly encourage participation on the coring expedition to offshore Brazil in February-March 2010. The successful candidate will also have opportunity to participate in other ongoing research projects on paleoclimate and biogeochemistry, and is encouraged to develop new lines of research topics of mutual interests.

Interested candidates for this position must apply both online at <https://employment.usf.edu> and directly to davidh@marine.usf.edu. Please send a curriculum vitae, names of three referees, a statement of qualifications and research interests. For information regarding this available position, please contact **Dr. David Hollander, Associate Professor, 727-553-1019** dhollander@marine.usf.edu. Please also visit www.marine.usf.edu.

VISITING RESEARCH ASSOCIATE/ FISH POPULATION DYNAMICS

We are seeking an outstanding scientist with a recent doctorate in marine science, oceanography or a closely related field relevant to fish population dynamics, fisheries oceanography, marine and estuarine sciences, or marine ecology. Strong pedagogical skills, experience organizing and participating in interdisciplinary fishery and oceanographic research, and synthesis of ecosystem datasets is essential. Demonstrated ability to apply biological, climate and oceanographic data to fish population dynamics using modern/emerging multispecies/ecosystem studies and statistical modeling is preferred. The successful applicant will be expected to teach graduate-level courses in fish population dynamics.

Advanced knowledge of the theory, principles and practices of fish population ecology, stock assessment, fisheries management, and a working knowledge of the physical and biogeochemical processes driving primary production, zooplankton dynamics, and food-web regulation is desired. The ability to develop and refine predictive population monitoring and assessment techniques (e.g., factors influencing year-class strength) of a detail necessary to evaluate direct effects of human actions and climate change is desired.

Interested candidates should apply online at <https://employment.usf.edu>. Please submit a statement of research interests, include a cover letter, curriculum vitae and contact information for three referees, no later than December 1, 2009. For information regarding this available position (14069), please contact **Dr. Ernst Peebles, Associate Professor, 727-553-3983** epeebles@marine.usf.edu. Please also visit www.marine.usf.edu.

USF is an Equal Opportunity/Equal Access University.



• TAMPA • ST. PETERSBURG • SARASOTA • MANATEE • POLYTECHNIC

Program in Science Technology and Environmental Policy at Princeton University - 2010-2011 - Postdoctoral Fellowship Program

The Program in Science, Technology and Environmental Policy (STEP) at Princeton University's Woodrow Wilson School of Public and International Affairs (Michael Oppenheimer, Director) announces its 2010-2011 Postdoctoral Fellowship Program. STEP will award one-year research positions (with the possibility of renewal for a second year) to eligible, talented researchers. These awards are designed to promote basic policy-relevant research under the supervision of one or more STEP faculty members. STEP faculty are offering fellowship opportunities in the following areas of interest:

- **David Wilcove:** (1) conservation of migratory animals (2) impacts of logging and agriculture on biodiversity in Southeast Asia
- **Michael Oppenheimer:** (1) modeling the role of learning in decisions where structural model error is a key concern. This work will be coordinated with ongoing case studies of actual scientific learning coupled to policy decisions. For further insights on this project, see <http://www.springerlink.com/content/7uw8150573197707/fulltext.pdf>. (2) analysis of paleoclimate proxies for sea level, ice extent, and temperature to improve the use of analogs in forecasting future sea level rise. This project focuses particularly, but not exclusively, on proxies from the Last Interglacial.
- **Denise Mauzerall:** research interactions between air pollution and climate change including chemical transport and climate modeling, analysis of potential co-benefits and technical and policy options for mitigation.

The Postdoctoral Fellows Program is open to all regardless of citizenship, but requires a completed doctorate and does not support work towards the completion of a degree. STEP fellows will be eligible for salary and full employee benefits in accordance with University guidelines.

Applicants should send a CV and a cover letter describing their areas of expertise and interest via <https://jobs.princeton.edu> (use requisition number 0900477). The review process will commence immediately and continue until positions are filled. For more information about applying to Princeton please link to: <http://web.princeton.edu/sites/dof/ApplicantsInfo.htm>. Candidates may choose to complete the "Invitation to Self-Identify" form <http://web.princeton.edu/sites/dof/forms/PSoftSelfID.pdf>. Providing the self-identification information is completely voluntary and declining to submit the information will not adversely affect your candidacy.

Princeton University is an Equal Opportunity Employer and complies with applicable EEO and affirmative action regulations.



Tenure-track Position

The Department of Anatomy and Neurobiology of the University of Puerto Rico (UPR) School of Medicine is open for recruitment in a tenure-track position at the Assistant Professor level. Applicants must have a Ph.D., postdoctoral experience, and a strong commitment to research and training of graduate students. Proven capacity to establish and/or maintain an independent externally funded research program is required. Field of research should fall within the mission of the National Institutes of Neurological Disorders and Stroke (NIH NINDS). Future teaching responsibilities may be in neuroscience, histology/cell biology, embryology and/or gross anatomy, for medical/graduate students (in either English or Spanish).

Review of applications will continue until the position is filled. To apply, send curriculum vitae, cover letter, separate statements of research interests/plans and teaching experience, and names/contact information of three references to: **Dr. Maria A. Sosa, Chair, Department of Anatomy and Neurobiology, School of Medicine, University of Puerto Rico, PO Box 365067, San Juan, PR 00936-5067.**

Further information:
maria.sosa@upr.edu;
<http://www.md.rcm.upr.edu/anatomyneurobiology/>

The UPR School of Medicine is LCME accredited and is an Equal Opportunity/Affirmative Action Employer.

COLUMBIA UNIVERSITY Tenured and Tenure-Track Faculty Positions Department of Electrical Engineering

The Department of Electrical Engineering at Columbia University in the City of New York invites applications for faculty positions.

Appointments at all levels, including assistant professor, associate professor, and full professor, will be considered. Priority cross-cutting themes include Energy, Engineering Biology, Information Technology, Advanced Computing, and Nanoscience/technology. Candidates who work in specific technical areas, including, but not limited to, computer engineering, networking and communications, and systems biology, with research programs that can significantly impact the above priority themes are particularly welcome to apply. Candidates doing other research at the interface of electrical engineering and the life sciences, physical sciences, and chemistry are also encouraged to apply.

Candidates must have a Ph.D. degree and are expected to establish a strong research program and excel in teaching both undergraduate and graduate courses.

Applicants should apply online on the Department Web site at:

<https://academicjobs.columbia.edu/applicants/Central?quickFind=52158>

The position will close no sooner than December 15, 2009, and will remain open until filled.

Columbia University is an equal opportunity/affirmative action employer.

NATIONAL RESEARCH COUNCIL OF THE NATIONAL ACADEMIES Research Associateship Programs

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- Awards for independent research at over 100 participating laboratory locations
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- Annual stipend \$42,000 to \$75,000 - higher for senior researchers
- Relocation, professional travel, health insurance
- Annual application deadlines Feb. 1, May 1, Aug. 1, Nov. 1
- Open to US and non-US citizens

Detailed program information, including instructions on how to apply, is available on the NRC Web site at:
www.national-academies.org/rap

Applicants must establish dialogue with Advisors at the Lab prior to application deadline.

Questions should be directed to the:
National Research Council

TEL: (202) 334-2760

E-MAIL: rap@nas.edu

Qualified applicants will be reviewed without regard to race, religion, color, age, sex or national origin.

THE NATIONAL ACADEMIES
Advisers to the Nation on Science, Engineering, and Medicine

Pushing forward the frontiers of Plant and Microbial Biology...



DIRECTOR AND CHIEF EXECUTIVE, THE JOHN INNES CENTRE

Norwich Research Park, Norwich, UK



The JIC's mission is to conduct high quality fundamental and strategic research relating to the understanding and exploitation of plants and microbes, with special emphasis on yield and productivity, quality and valuable products, and environmental interactions.

A scientist with an established international reputation in research relevant to the JIC's mission, you will be responsible for promoting and furthering the Institute's national and international profile as a globally renowned scientific research establishment in plant and microbial biology.

With proven management skills you will provide inspirational and supportive leadership and vision to internationally recognised scientists as well as an attractive environment for the development of early career scientists from across the world. You will ensure that the JIC continues to deliver cutting-edge research of the highest quality whilst ensuring significant outputs to its range of public and private stakeholders, making a notable contribution to the global challenges facing society across a range of areas including food security, living with environmental change, healthy ageing, the control of infectious diseases and the development of industrial products from plants.

As Director and Chief Executive of JIC, you will be responsible for all aspects of the Institute's business including maintaining its long term sustainability, and playing a pivotal role in the development and implementation of the vision for the Norwich Research Park (NRP) with the John Innes Foundation, University of East Anglia (UEA), Institute of Food Research, The Genome Analysis Centre, Sainsbury Laboratory, and the Norfolk and Norwich Hospital, all of which are based on the NRP. This includes the existing Earth and Life Systems Alliance (ELSA) programme between JIC and UEA.

It is expected that you will wish to maintain active research interests and support will be provided in order to allow you to do this.

An attractive salary plus performance related bonus is available commensurate with the level and the strategic importance of the post.

Further information, including instructions on how to apply, may be obtained from BBSRC's recruitment consultants, Carbon-NfP, who would be happy to provide further written information and/or to have an informal and confidential telephone discussion. Please contact Sharon Taylor by email sharon.taylor@carbon-nfp.com in the first instance.

The closing date for applications is Friday, 27 November 2009.

Carbon-NfP, 17 Russell Court, Woburn Place, London WC1H 0LL.

BBSRC welcomes applications from all sections of the community irrespective of race, ethnic origin, religion or belief, sexual orientation, disability, age or gender. As users of the disability symbol, we guarantee to interview all disabled applicants who meet the minimum criteria for the vacancy.



www.carbon-nfp.com



4 Ecological Post-Doctoral Research Appointments

UNIVERSITY OF QUEENSLAND, BRISBANE, AUSTRALIA AND UNIVERSITY OF EXETER, UK

- 1) Modelling of climate change impacts and accretion of Caribbean coral reefs (4 years). Post based at University of Exeter (UoE) but seconded to the University of Queensland (UQ). This position is part of a collaborative project with Caribbean partners and involves extensive travel in the region. (Post reference: N2381). Salary in the range £26,391 - £31,513 pa depending on experience. Post available from 1 March 2010.
- 2) Spatially-realistic modelling of coral and fish metapopulation dynamics (3 years). Based at the UoE but seconded to UQ. (Post reference: N2307). Salary in the range £26,391 - £30,594 pa depending on experience. Post available from 1 January 2010.

In connection with the vacancies at the University of Exeter the following posts will be made available by the University of Queensland:

- 3) Spatial modelling of climate change impacts on dynamics of Pacific coral reef ecosystems (5 years). Position funded at University of Queensland (UQ) (Post reference: UQ1).
- 4) Coral reef field ecologist with interests in reef resilience and ecosystem services (5 years). (Post reference: UQ2). Salary for posts 3 and 4 is AU\$61,399 pa plus generous superannuation package (additional 17%). Both posts are available from 1 April 2010.

Applicants of any nationality are eligible to apply for these positions. A PhD and appropriate publication track record are required for all posts. A background in ecological modelling is required for posts 1, 2 and 3 though this does not have to involve coral reefs or marine ecosystems. For post 1, a background in bioerosion and reef accretion/calification would be highly desirable. The field ecologist should already have developed a track record in coral reef ecology. Research interests can vary and may include coral population dynamics, herbivory, algal dynamics, population connectivity or reef fish biodiversity. The long tenure of these positions make them an excellent opportunity to develop an impressive track record in ecology and marine conservation.

For further information about these posts contact Professor Peter Mumby at the University of Exeter, email p.j.mumby@ex.ac.uk

To apply:

Please send your CV and covering letter with the contact details of three referees to Professor Peter Mumby, School of Biosciences, Hatherly Laboratories, University of Exeter, Prince of Wales Road, Exeter EX4 4PS, UK (e-mail p.j.mumby@ex.ac.uk) quoting the relevant job reference number.

The closing date for completed applications is 25th November 2009.

The University of Exeter is an equal opportunity employer and promotes diversity in its workforce and, whilst all applicants will be judged on merit alone, is particularly keen to consider applications from groups currently underrepresented in the workforce.



THE UNIVERSITY
OF QUEENSLAND
AUSTRALIA



Group Leader in Zebrafish Development and Regeneration, Australian Regenerative Medicine Institute

Applications are invited for a mid career, senior research scientist for a Group Leader position in the Eva and Les Erdi Zebrafish Research Group. This research group will focus on zebrafish genetics and development and the successful applicant will be a dynamic, independent scientist with an outstanding record of research success in applying zebrafish genetics to fundamental questions of developmental and/or regenerative biology. They will join an exciting new research Institute environment and will be expected to initiate and maintain strong research programs synergizing with the Monash research community and to take an active part in collaborative research. Applicants who use zebrafish to study aspects of regenerative biology are specifically encouraged to apply.

The **Australian Regenerative Medicine Institute (ARMI)** is a state-of-the-art regenerative medicine research facility based at Monash University, Melbourne, Australia. Monash is a young, dynamic and internationally recognized university with an established reputation for providing excellence in biomedical research, training and education in campuses around the world.

Benefits:

- Competitive salary with 17% superannuation plus generous start up package
- State-of-the-art laboratories and access to leading edge core research facilities including "Fish-core" a newly built 7000 tank zebrafish facility
- A range of professional development programs, support for research, study and overseas work, generous maternity leave and flexible work arrangements.

Commencement Date: Mid 2010

Duration: The appointment will be for a period of five years

Further information about ARMI and a position description can be obtained at:

<http://www.med.monash.edu.au/armi/>

Questions about this position should be directed to: Prof. Peter Currie, Deputy Director, peter.currie@armi.monash.edu.au

To apply, please email a cover letter, CV (in English), three letters of recommendation and a summary of present and future research interests, to positions@armi.monash.edu.au by **13 December 2009**, quoting ref. no. A0910345 in the subject line.



The California Nanosystems Institute (CNSI) at the University of California-Santa Barbara is pleased to announce the competition for the ELINGS PRIZE FELLOWSHIPS in EXPERIMENTAL SCIENCE

These prize postdoctoral fellowships provide a salary of \$60,000/yr for two years, renewable for a third, along with benefits and research funds. Successful applicants will work with experimental CNSI faculty; applicants should indicate the experimental group(s) with which they would prefer to work. See www.cnsi.ucsb.edu/fellowships.

Applicants holding a PhD in science or engineering should apply at <https://fellow.cnsi.ucsb.edu/> by submitting a cover letter, curriculum vitae, a one-page research proposal, and arranging for 3 supporting letters. The deadline for applying is **December 1, 2009**.

The University of California is an Equal Opportunity/Affirmative Action Employer.



國家衛生研究院
National Health Research Institutes

Immunology Research Center National Health Research Institutes (NHRI) Taiwan

The newly established Immunology Research Center at the National Health Research Institutes (NHRI) in Taiwan invites applications for multiple tenure-track/tenured faculty positions at the rank of **Assistant, Associate or Full Investigator** (the equivalents of Assistant, Associate, and Full Professor). Highly qualified candidates are sought with research interests in all areas of **Immunology and Signal Transduction**, especially in the areas of cell signaling and gene regulation, innate immunity, immune tolerance, autoimmunity, and cancer immunology. Applicants should have a Ph.D. and/or M.D. degree as well as extensive postdoctoral experience. Selection will be based on excellence in research and the potential to maintain an outstanding research program. Investigators will have the opportunity to train graduate students from several affiliated universities. A generous annual intramural support will be provided.

Applicants should send curriculum vitae, description of research accomplishments and future objectives, and three reference letters to:

Faculty Search Committee
Immunology Research Center
National Health Research Institutes
35 Keyan Road, Zhunan Town,
Miaoli County 35053, Taiwan

Review of credentials is ongoing and will continue until the positions are filled. Further information can be obtained from Ms. Yu-Feng Huang at kitty01@nhri.org.tw.



PROTEOMICS FACULTY POSITION

The Department of Genome Sciences at the University of Washington School of Medicine in Seattle invites applications for a faculty position at the rank of **ASSISTANT PROFESSOR** working in the field of proteomics on protein composition, function, dynamics, localization and interactions. Exceptional candidates may be considered at the rank of **ASSOCIATE PROFESSOR**.

Primary emphasis is on the establishment of an outstanding independent research program in proteomics, as well as participation in the teaching and service responsibilities in the Department of Genome Sciences. The position also provides association with the Proteomics Resource, which offers access to state-of-the-art equipment and facilities, as well as opportunities to establish collaborations with laboratories throughout the University of Washington and to play a leadership role in the University's wider proteomics program.

The Proteomics Resource facility occupies almost 7000 sq. ft. of space on the School's new Lake Union Campus and has obtained substantial long-term financial support of its mission, allowing for equipment acquisition and renewal, development of computing resources and recruitment of key personnel.

Applications received by **December 15, 2009**, will receive consideration. Thereafter, applications will be reviewed upon receipt until the position is filled. Applicants should hold a Ph.D. or M.D. degree. Candidates should email their curriculum vitae and statement of research and teaching interests, and arrange to have three signed letters of reference sent to: **faculty-search@gs.washington.edu**.

More information about the Department and the Proteomics Resource is available at <http://www.gs.washington.edu> or <http://proteomicsresource.washington.edu>.

The University of Washington is an Affirmative Action, Equal Opportunity Employer. The University is building a culturally diverse faculty and staff and strongly encourages applications from women, minorities, individuals with disabilities and covered veterans. The University of Washington Faculty engage in teaching, research and service.



CALIFORNIA STATE UNIVERSITY, FRESNO
Jordan College of Agricultural Sciences and Technology
Department of Viticulture and Enology
Chair of the Department of Viticulture and Enology and
Director of the Viticulture and Enology Research Center
(Associate Professor/Professor)



California State University, Fresno is one of 23 campuses in the California State University System. Metropolitan Fresno, with a multi-ethnic population of over 600,000, is located in the heart of the San Joaquin Valley on the western edge of the Sierra Nevada Mountain Range. The Jordan College of Agricultural Sciences and Technology's **Department of Viticulture and Enology (DVE)** and **Viticulture and Enology Research Center (VERC)** provides world-class grape and wine education, research, and outreach programs. Our faculty and practical hands-on teaching and research programs are nationally and internationally recognized.

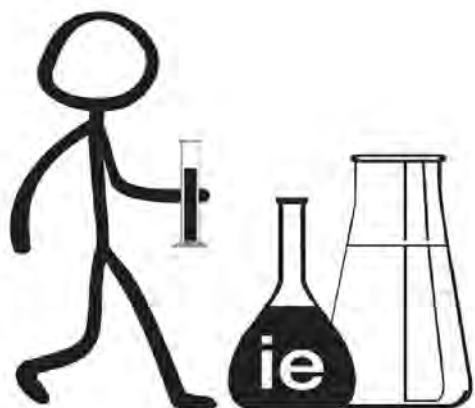
Position: AY: 2010-2011. 12-month assignment with tenure: **Chair** of the Department of Viticulture and Enology and **Director** of Viticulture and Enology Research Center. The appointment typically will be 25% administrative, at least 65% research and outreach, and approximately 10% teaching. Professionalism, collegiality, and integrity are essential traits. The successful candidate will be expected to provide leadership of the DVE and VERC for the full range of responsibilities necessary to administer, develop, and operate programs including but not limited to: (1) planning and administering the academic and research programs, including the enhancement of instruction and building research capacity; (2) develop strong and effective liaisons with all branches of the viticulture and enology industries of California's San Joaquin Valley; (3) formulating and implementing strategic plans and budgets through consultations with administrators, faculty, staff and industry; and (4) effectively representing the DVE and VERC. Salary placement depends upon academic preparation and professional experience.

Qualifications: An earned doctorate (Ph.D.) from an accredited institution, evidence of ability to plan, budget, and manage an academic department in a consultative and collegial style required. Candidates are expected to demonstrate evidence of research, scholarly and teaching activity commensurate with the rank of Associate Professor or Full Professor with tenure. Appointment at the rank of Professor will require a record of outstanding teaching, scholarly work, and/or professional activities. Must have the ability to work effectively with faculty, staff, and students from diverse ethnic, cultural, and socioeconomic backgrounds. Candidates having experience and familiarity with California grape and wine industries, experience in warm weather viticulture or enology, and the ability to use technology to enhance instruction preferred.

Open until filled: For full consideration, applicants are encouraged to have all application materials on file by **February 5, 2010**. Contact: **Dr. Roy Thornton, Search Committee Chair**; Ph: (559) 278-7112; Fax: (559) 278-4795; Email: rthornto@csufresno.edu. Apply online: <http://jobs.csufresno.edu>. Full announcement: <http://jcast.csufresno.edu/ve>.

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GRADUATE PROGRAM



Discover the formula for success

Master in Biotechnology Management

Recent advances in life sciences have brought about a revolution in the biotechnology industry. To face these new challenges and meet the resulting business opportunities, IE Business School offers an innovative and challenging Master's program in Biotechnology Management, which combines general business knowledge with specialized industry know-how.

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 Admissions contact: biotech@ie.edu

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www.ie.edu/business



University of Connecticut Health Center

Faculty Position in Retinal Stem Cells and Retinal Degenerative Diseases

The Department of Neuroscience at the University of Connecticut School of Medicine is seeking a highly qualified individual with an outstanding background in retinal stem cells and retinal degenerative diseases. The John A. and Florence Mattern Solomon Chair in Vision Biology and Eye Diseases will partially support the work of the new faculty member. The applicant will be a Ph.D. and/or M.D. with experience in stem cells and an interest in applying that expertise to novel therapies of retinal diseases including macular degeneration. The successful candidate will have very strong research credentials including a history of major publications and well-funded research, along with experience teaching. The stem cell expertise at UCHC, coupled with the state-of-the-art imaging facilities available in the Center for Cell Analysis and Modeling and our Translational Genomics Core, make UCHC an excellent home for this type of research, which is an area of intense interest at the National Eye Institute.

Applications are invited for this tenure track position at the Associate or Full Professor level. Faculty will enjoy superb resources including a generous start-up package as well as state-of-the-art core facilities for mouse transgenics and ES cell manipulation, cellular electrophysiology, microarrays and next-generation sequencing, flow cytometry, confocal microscopy and fluorescence imaging. The successful candidate will be expected to expand upon his/her funded independent and innovative research program, and to actively contribute to a rich scientific environment and expertise in the Neuroscience Department.

Candidates are invited to visit the departmental web page (<http://neuroscience.uchc.edu>) and should apply by submitting electronically a curriculum vita, a one page description of research goals, and a pdf of a current published paper. The candidate should also solicit letters from three references. Applications and letters should be sent via the University of Connecticut Health Center Employment Services website, <https://jobs.uchc.edu>, search code 2010-374.

UCHC is an Equal Opportunity Employer M/F/V/PwD



University of Connecticut Health Center

Faculty Position in Biomolecular NMR

The Department of Molecular, Microbial, and Structural Biology at the University of Connecticut Health Center invites applications for a junior faculty position in biomolecular NMR. We seek candidates who complement existing strengths (<http://mmsb.uchc.edu>, <http://structbio.uchc.edu>, <http://sb.uconn.edu>) and we particularly encourage applications from women and minority candidates. The successful candidate will be expected to establish a robust research program and play a leadership role in departmental, campus, and university-wide research and teaching in structural biology. Candidates will be expected to demonstrate academic achievement and promise as an independent investigator. UCHC offers outstanding NMR resources, including spectrometers operating at 500, 600, and 800 MHz (all with cryogenic probes), as well as a well-equipped biophysical core facility.

The closing date for receipt of applications is January 5, 2010. Applicants should submit a letter of application, curriculum vitae, research plan and statement of teaching interests, and names (with address and e-mail address) of at least three references. Applications should be submitted via the University of Connecticut Health Center Employment Services website, <https://jobs.uchc.edu>, search code 2010-377.

UCHC is an Equal Opportunity Employer M/F/V/PwD



University of Connecticut Health Center

Tenure Track Faculty Position in Geriatric or Aging Research

The University of Connecticut Center on Aging is a multidisciplinary center with a \$30 million research portfolio supporting clinical, basic and health outcome/population studies designed to improve independence, function and quality of life in older adults and their families (<http://www.uconn-aging.uchc.edu/>). Applications for a tenure track position in these areas are welcome at any level, but investigators with established funded research programs are especially encouraged to apply. Position requires MD, Ph.D. or equivalent degree, as well as a track record of research and funding relevant to frailty and disability associated with aging. Areas of particular interest include, but are not limited to: exercise, hormonal, behavioral or health service interventions designed to improve function and clinical outcomes; studies addressing the role of inflammation and oxidative stress; immunosenescence and stem cell biology.

Faculty will enjoy superb resources including a generous start-up package, state-of-the-art institutional research core facilities (<http://uchc.edu/hc/research.html>) and many collaborative opportunities in aging research (<http://www.agingnet.uchc.edu/>). Participation in the training of graduate students, postdoctoral and clinical fellows, and undergraduates is encouraged.

Candidates should apply by submitting a curriculum vita and the names of 3 references via the University of Connecticut Health Center Employment Services website, <https://jobs.uchc.edu>, search code 2010-375.

UCHC is an Equal Opportunity Employer M/F/V/PwD

NYU

Langone Medical Center

www.apply.nyumc.org

DIRECTOR, NEUROSCIENCE INSTITUTE NYU School of Medicine & its Affiliated Academic Medical Centers

The NYU School of Medicine announces its search for the Director of the Neuroscience Institute. The Dean and faculty consider this an exceptional opportunity to lead a preeminent institute in the City of New York in close collaboration with the other schools and colleges of New York University.

The Director of the Neuroscience Institute will head research and recruitment efforts at our newly founded Neuroscience Institute. The successful candidate will have a PhD and/or MD and will have demonstrated leadership experience in a university, medical center or research-institute setting, with a distinguished record of scientific achievements and a vision for the future of neuroscience.

Applications and nominations with accompanying curriculum vitae should be sent electronically, for confidential review by the search committee, to: **Rebecca Elwork, M.H.S.A., Project Manager for Education, Faculty and Academic Affairs, Rebecca.Elwork@nyumc.org**.

The NYU School of Medicine was founded in 1841 and is an Equal Opportunity, affirmative action employer and provides a drug-free and smoke-free workplace.



Postdoctoral Positions Initiative for Bioinformatics and Evolutionary Studies (IBEST)

The Initiative for Bioinformatics and Evolutionary Studies at the University of Idaho is seeking four postdoctoral scientists with expertise in bioinformatics and computational biology to conduct research on the human microbiome and viral host switching. These postdoctoral scientists will play a leading role in developing new, computationally intensive, tools for such analyses, and interfacing with the experimentalists responsible for generating the data. These postdoctoral scientists will also be part of the Initiative for Bioinformatics and Evolutionary Studies (IBEST) group at the University of Idaho (<http://www.ibest.uidaho.edu/ibest/index.html>); a dynamic and interdisciplinary research group with excellent research resources.

HUMAN MICROBIOME

Two postdoctoral scientists will join interdisciplinary, multi-institutional teams of investigators to conduct research to develop and apply statistical methods for the analysis of data from studies on the microbiome of the human vagina. In these projects state-of-the-art genomic technologies will be used to define the dynamics of vaginal bacterial communities using data on the metagenome, metatranscriptome, metabolome, community species composition, as well as clinical and behavioral metadata. Statistical models will then be used to define the molecular events that lead to bacterial vaginosis or increase risk to sexually transmitted diseases. One project is an NIH funded Human Microbiome demonstration project, while the second is within the Eco-Pathogenomics of Chlamydial Reproductive Tract Infection (EPCRTI), an NIH funded Sexually Transmitted Infections Cooperative Research Center. The successful candidates will work under the supervision of **Dr. Zaid Abdo** (Departments of Mathematics and Statistics) and **Dr. Larry Forney** (Department of Biological Sciences).

ADAPTIVE EVOLUTION OF VIRUSES

Two postdoctoral scientists will work as part of an interdisciplinary team to combine mathematical theory with empirical studies that use a bacteriophage model system to explore general rules of viral adaptation. This will involve the use of statistical techniques and computational modeling informed by rigorous experiments to predict adaptive evolution at the molecular level. One postdoctoral scientist will focus mainly on statistical and mathematical modeling while the other will focus on empirical dissection of adaptive evolution and the molecular level. The successful candidates will work under the supervision of **Dr. Paul Joyce** (Department of Mathematics) and **Dr. Holly Wichman** (Department of Biological Sciences).

Qualifications: The successful candidates will have a Ph.D. degree in bioinformatics and computational biology, statistics, biostatistics or a related discipline. They will also have a strong record of research accomplishments, including multiple publications in peer reviewed scientific journals, excellent oral and written communication skills, and the ability to work effectively as part of an interdisciplinary team. A basic understanding of the ecology and evolution of microbial communities and knowledge of genetics and molecular evolution is desirable. Other requirements are listed in the position description posted on the University of Idaho Human Resources website (<http://www.hr.uidaho.edu/default.aspx?pid=35496>). Qualified candidates should apply no later than **November 30, 2009** via the Human Resources website. Applications should include a cover letter, curriculum vitae, and three letters of recommendation.

The University of Idaho is an Equal Opportunity/Affirmative Action Employer.

SOUTHERN ILLINOIS UNIVERSITY EDWARDSVILLE

ASSISTANT PROFESSOR - ANATOMIST

Description of Duties: Applicants should have broad training in human and/or vertebrate anatomy. The successful candidate will share responsibility for teaching a human anatomy course for majors, human anatomy and physiology course for non-majors, and advanced courses in their specialty area. Expertises in areas of interest to pre-health professional students (e.g., development biology, histology, neurobiology, and endocrinology) are desirable. The successful candidate will also be expected to participate in teaching introductory courses for biology majors and non-majors, and should exhibit potential for independent and innovative research involving Master's and undergraduate students.

Qualifications Required: A PhD in biology or related field. Relevant Post-doctoral teaching and research experience preferred.

Closing Date for Applications: Review of applications will begin on **December 1, 2009**, and continue until position is filled.

Submit: To apply, send a letter of application with a statement of teaching philosophy, statement of research interests, *curriculum vitae*, copies of official transcripts, three letters of reference, and no more than three reprints to:

Chair, Physiologist Search Committee
Department of Biological Sciences
Southern Illinois University Edwardsville
Campus Box 1651S
Edwardsville, IL 62026-1651

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JOHN TEMPLETON FOUNDATION

EXECUTIVE VICE PRESIDENT WEST CONSHOHOCKEN, PENNSYLVANIA

The John Templeton Foundation invites nominations and applications for the position of Executive Vice President.

Founded in 1987 by legendary global investor and philanthropist, Sir John Templeton, the mission of the \$1.5 billion asset Foundation is to serve as a philanthropic catalyst for discovery into areas engaging in life's biggest questions. JTF provides grants and programs that challenge religions to realize how much they still need to learn and understand, and to embrace a culture of discovery that absorbs new insights and generates spiritual progress, particularly, but not exclusively, through scientific research.

The Executive Vice President will report directly to the President, and as his deputy, will play a major role in all aspects of the Foundation's activities. The successful candidate will have substantial intellectual credentials relevant to JTF's core program areas (Natural Sciences, Human Sciences, Philosophy and Theology, Character Development, Freedom and Free Enterprise, Gifted Education and World Religions) and will have a background in scientific research or within a multifaceted scientific organization where innovative global thinking is essential. The Executive Vice President's ability to create and maintain networks of academics and others who resonate with the Foundation's mission will be a critical component of the Executive Vice President's core responsibilities.

More information about the Foundation can be found on its website www.templeton.org.

Please send all nominations or applications to **Susan Meade** and **Jane Phillips Donaldson** at JTF@PhillipsOppenheim.com.

The John Templeton Foundation is an Equal Opportunity Employer.

POSITIONS OPEN



Division of Gastroenterology, University of Maryland, Baltimore has NIH-funded **POSTDOCTORAL/JUNIOR FACULTY POSITION** available to study muscarinic receptors and ligands in colon cancer with emphasis on cell signaling. Ph.D., expertise in cell and molecular biology, strong motivation, and *U.S. citizenship/permanent residency* required. Salary based on experience. Send cover letter, curriculum vitae, three letters of reference electronically to: **Dr. Jean-Pierre Raufman, e-mail: jraufman@medicine.umaryland.edu**. The University of Maryland, Baltimore is an Equal Opportunity/Affirmative Action Employer. Minorities, women, veterans, and individuals with disabilities are encouraged to apply.

FACULTY POSITION

The Department of Molecular Physiology and Biophysics at the University of Vermont is seeking to recruit a **CELL BIOLOGIST/BIOPHYSICIST** at the **ASSISTANT PROFESSOR** level on the tenure-track, although **ASSOCIATE** and **FULL PROFESSOR** candidates will be considered.

The Department has significant strength in protein structure and function with emphasis on contractile and cytoskeletal proteins. The ideal candidate will complement existing expertise in molecular biology, single molecule biophysics, and structural biology. The candidate will be expected to develop an independent, extramurally funded research program in cell biology with emphasis on mechanisms by which the cytoskeleton and molecular motors govern cellular function (e.g., cell signaling, intracellular transport, and cell division) which may be altered in human cancer and cardiovascular diseases.

The candidate must be willing to team-teach mammalian physiology in a medical and graduate school setting. Startup funds will be competitive and access provided to graduate students and postdoctoral fellows through departmental training grants.

Review of applications will begin immediately and continue until the position is filled. Include a resume, research plan, teaching experience, and the names of three references whose letters must be received prior to review of your application.

Address all inquiries and materials to:

Dr. Robert Low
Chair Faculty Search Committee
Department of Molecular Physiology and Biophysics
Health Science Research Facility
149 Beaumont Avenue
Burlington, VT 05405-0075 U.S.A.
E-mail: bob.low@uvm.edu

Or apply online at website: <http://www.uvmjobs.com>.

The University of Vermont is an Equal Opportunity/Affirmative Action Employer. Applications from women and people from diverse racial, ethnic, and cultural backgrounds are encouraged.

THE HEISER PROGRAM FOR RESEARCH IN LEPROSY AND TUBERCULOSIS

Beginning **POSTDOCTORAL RESEARCH FELLOWSHIPS** in leprosy and tuberculosis research available at a stipend level of \$40,000 a year for two years, and **RESEARCH GRANTS** in leprosy usually in amounts up to \$50,000 for one year.

Applicants should have M.D., Ph.D., or equivalent degree. Application deadline March 1, 2010, for awards to be activated August through December 2010. For information and forms, see website: <http://www.NYCommunitytrust.org>. Click on Grant Seekers and select Requests for Proposals from the drop-down menu. Click on The Heiser Program in the list down the right side of your screen to access the full request for proposals and application forms. For questions or problems, e-mail: lm@nyct-cfi.org.

POSITIONS OPEN



The University of Texas at Austin's Marine Science Institute and Department of Marine Science invite applications for a tenure-track/tenured **ASSISTANT** or **ASSOCIATE PROFESSOR** (outstanding applicants at the rank of **FULL PROFESSOR** will also be considered) based at the Marine Science Institute in Port Aransas, Texas. We seek an experimentalist who will study physiological processes in juvenile, larval, or adult marine fishes of commercial or recreational importance using molecular techniques to investigate basic questions in growth, condition, nutrition, or reproduction. We seek candidates who would benefit from excellent fish culture facilities at our Fisheries and Mariculture Laboratory.

Details of the position, the Institute, and application procedure are available at websites: <http://www.utmsi.utexas.edu/hr> and <http://facultyjobs.utexas.edu>.

The University of Texas at Austin values diversity and is committed to Affirmative Action and Equal Opportunity. Women and minorities are encouraged to apply. Background checks conducted on applicant selected.

ASSISTANT/ASSOCIATE PROFESSOR in the Anatomical Sciences University of North Texas Health Science Center

The Department of Cell Biology and Anatomy at the University of North Texas Health Science Center (UNTHSC) at Fort Worth invites applications for gross anatomists with a minimum of two to three years of teaching responsibilities in one or more of the following areas: medical human gross anatomy (cadaver-based) and neuroanatomy. The successful applicant(s) must have a Ph.D., D.O., M.D., or equivalent degree and experience in teaching a clinically oriented human anatomy course with human cadaver dissection. The anatomical sciences at UNTHSC are team taught, and the applicants will be expected to contribute to both lecture and laboratory instruction in these disciplines. Other teaching responsibilities may include lecture and laboratory instruction in the prosection-based physician assistant (PA) human anatomy program, and lectures in medical histology and embryology. Current funding is not a requirement; however, candidates will be expected to develop a sustainable research program in clinical human anatomy, biomedical science/basic science, anatomical education, and/or science educational outreach programs. Candidates should apply and submit their curriculum vitae through the Human Resource Services online applicant tracking system at website: <http://www.unthscjobs.com> along with a letter of interest, summary of past research accomplishments, future research plans, current funding, past teaching experiences and teaching philosophies, and/or interests. Three letters of reference should be mailed to: Search Committee for Anatomy Positions, c/o Department of Cell Biology and Anatomy, University of North Texas Health Science Center, 3500 Camp Bowie Boulevard, Fort Worth, TX 76107.

The University of North Texas Health Science Center at Fort Worth is an Equal Opportunity/Affirmative Action Institution.

TWO TENURE-TRACK ASSISTANT PROFESSORS

Developmental Biology and Animal Biology

Applications are encouraged from candidates with a Doctorate and at least two years of research experience past the Doctorate in either (1) developmental biology or (2) animal biology. Successful candidates are expected to participate in undergraduate and graduate teaching and to develop an active research program at the University of Northern Colorado. For details see website: <http://www.unco.edu/nhs/employment.html>. For questions, contact the search chairs at (1) e-mail: gregory.dekrey@unco.edu or (2) e-mail: stephen.mackessy@unco.edu. Review of applications begins January 10, 2010, and will continue until the positions are filled.

UNC is an Affirmative Action/Equal Opportunity Employer.

POSITIONS OPEN



The Department of Biology and Chemistry at Azusa Pacific University, a Christian university in southern California, announces an opening for a **FULL-TIME PROFESSOR OF BIOLOGY** with an expertise in the area of anatomy. Candidates should have a Ph.D., M.D., or D.P.T., a strong commitment to undergraduate teaching, experience using cadavers in teaching anatomy, a strong commitment to research in an undergraduate setting, and a demonstrated, vibrant Christian faith compatible with the mission of Azusa Pacific University. Postdoctoral experience is preferred. Responsibilities begin in mid August 2010. Academic rank and compensation will be based on degrees and experience. Review of completed applications will continue until the position is filled. A more complete description of the position, its requirements, and the application process is available at website: <http://www.apu.edu/provost/employment/positions/>.

Azusa Pacific University does not discriminate on the basis of race, color, national origin, gender, age, disability, status as a veteran, or other characteristics protected by law in its programs, policies, or procedures. Minorities and women are urged to apply.

TRANSLATIONAL GENETICS FACULTY University of Washington Department of Medicine Division of Medical Genetics

The University of Washington invites applicants for full-time faculty positions at the rank of **ASSISTANT, ASSOCIATE, or full PROFESSOR** in the Division of Medical Genetics, Department of Medicine. Successful candidates will have an M.D. and/or Ph.D. and will be expected to carry out a research program in translational genomics. Candidates with a strong research background involving the identification and characterization of disease genes, or the application of genetic/genomic information toward the diagnosis or treatment of human disease, are especially encouraged to apply. Successful candidates will have opportunities to collaborate with other outstanding members of the faculty, and will have access to state-of-the-art research facilities and clinical research programs. The positions will remain open until filled.

Electronically send curriculum vitae to: **Medical Genetics Faculty Search c/o Sara Eisner, University of Washington, Division of Medical Genetics, e-mail: seisner@u.washington.edu**.

University of Washington faculty engage in teaching, research, and service.

The University of Washington is an Affirmative Action, Equal Opportunity Employer. The University is building a culturally diverse faculty and staff and strongly encourages applications from women, minorities, individuals with disabilities, and covered veterans.

SMITHSONIAN INSTITUTION FELLOWSHIP PROGRAM

GRADUATE STUDENT, PREDOCTORAL, POSTDOCTORAL, AND SENIOR FELLOWSHIPS in animal behavior, ecology, and environmental science, including an emphasis on the tropics; Earth sciences and paleobiology; evolutionary and systematic biology; history of science and technology. Tenable in residence at the Smithsonian facilities. Stipends and tenure vary. Awards are contingent upon the availability of funds.

Deadline: January 15 annually. Contact: **Office of Fellowships, Smithsonian Institution, Desk S, P.O. Box 37012, L'Enfant 7102 MRC 902, Washington, DC 20013-7012. Telephone 202-633-7070; e-mail: siof@si.edu; website: <http://www.si.edu/research+study>.**

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BODOSSAKI FOUNDATION



ANNOUNCEMENT

Applications are invited for four (4) post-doctoral scholarships established by the Bodossaki Foundation in memory of "Stamatis G. Mantzavinos" in Biomedical Sciences for the academic year **2010-2011** in the following fields:

1. IMMUNOBIOLOGY
2. GENETICS
3. CARCINOGENESIS
4. NEUROSCIENCES
5. PHARMACOLOGY

Scholarships will be offered to applicants of Greek nationality, parentage or descent. Applications' deadline: **30th November 2009**.

The full-length announcement (Greek version) can be obtained from the following link: <http://www.bodossaki.gr>. Applicants can also find a full-length announcement from the foundation's Secretariat at 23A Vas. Sofias Av., 3rd floor, Athens, 10674 during week days from 10 a.m. to 12 p.m.

For further information please contact: **+30210/3237-973 and +30210/323-7804**.

POSITIONS OPEN



ASSISTANT PROFESSOR. The University of Nebraska Medical Center (UNMC) College of Dentistry, Department of Oral Biology, Nebraska Center for Cellular Signaling (NCCS) has a full-time, tenure-track faculty position available July 1, 2010. The successful candidate is expected to establish a strong, externally funded research program in cell signaling aspects of cancer biology. Superb opportunities for research are available as the Department expands its research programs. Applicants with a research focus compatible with NCCS interests are encouraged to apply. Ph.D. or equivalent degree and postdoctoral experience required. Review of applications will continue until the position is filled. Applications are being accepted online at [website: jobs.unmc.edu](http://jobs.unmc.edu), position #3787. The University of Nebraska Medical Center is an Equal Opportunity/Affirmative Action Employer.

AQUATIC ECOLOGIST

The Department of Biology, Loyola University Chicago, invites applications for a tenure-track position in aquatic ecology at the **ASSISTANT PROFESSOR** level, beginning August 2010. Preference given to candidates working in stream ecology with research expertise complementing existing research strengths in the Department. For an overview of the Department, see our [website: http://www.luc.edu/biology/](http://www.luc.edu/biology/). We have modern laboratory facilities and a 2,100 square foot artificial stream facility with 48 racetrack-type streams. We are a large Department that services more than 1,500 undergraduate majors and 25 M.S. students. Candidates must have a Ph.D. and postdoctoral experience, and will be expected to establish a vigorous, externally funded research program involving undergraduates and M.S. students. Teaching responsibilities include general ecology, an advanced course in the candidate's area of specialization, or general biology. Candidates should complete the online application in full at [website: http://www.careers.luc.edu](http://www.careers.luc.edu) with cover letter, curriculum vitae, research plan, teaching philosophy statement, and letters from three references. Pending final funding, review of applications will begin on November 30, 2009, and continue until the position is filled. Written inquiries about the position can be sent to: **Aquatic Ecologist Search Committee, Loyola University Chicago, Department of Biology, 1032 West Sheridan, Chicago, IL 60660.** Loyola University Chicago is an Affirmative Action/Equal Opportunity Employer, and strives for diversity in its faculty, students, and staff; underrepresented minorities are particularly encouraged to apply.

EXPERIMENTAL PLANT ECOLOGIST. Tenure-track **ASSISTANT PROFESSOR** position available August 2010. Earned Doctorate in ecology, botany, or related discipline; research addressing plant responses to global climate change. Successful applicant must be qualified to teach field botany, introductory biology laboratories, and a variety of upper division courses in ecology. Candidate is expected to establish an active, externally funded research program involving graduate and/or undergraduate students. Finalists must successfully complete interview process and teaching demonstration. Mail one hard copy of all official university transcripts, statements of teaching and research philosophies, curriculum vitae, and three letters of recommendation to: **Dr. Harry M. Tiebout III, Department of Biology, West Chester University, West Chester, PA 19383** (no electronic applications). Review of completed applications begins on January 4, 2010, and continues until position is filled. For more details and full advertisement, visit [website: http://www.wcupa.edu/scripts/vacancies/v-list.asp](http://www.wcupa.edu/scripts/vacancies/v-list.asp); call telephone: 610-436-2726; or e-mail: htiebout@wcupa.edu. The filling of this position is contingent upon available funding. Affirmative Action/Equal Opportunity Employer. Women and minorities are strongly encouraged to apply. All offers of employment are subject to and contingent upon satisfactory completion of all preemployment background and consumer reporting checks.

POSITIONS OPEN

BIOMOLECULAR NMR SPECTROSCOPIST Indiana University

Indiana University has significantly expanded its nuclear magnetic resonance (NMR) capabilities with the installation of 600 and 800 MHz Varian spectrometers in the new Metabolomics and Cytomics Initiative Biomolecular NMR Laboratory in Simon Hall. We now seek to appoint a Biomolecular NMR Spectroscopist at the **ASSISTANT SCIENTIST** (research-track) level, effective April 1, 2010. Candidates must have a Ph.D. in chemistry, biochemistry, structural biology, or a related field; two years of postdoctoral experience or equivalent is desired. The successful candidate will have documented experience with the application and optimization of modern biological NMR spectroscopy to structural studies of proteins. Familiarity with partially aligned systems and nucleic acids is desirable. Experience with the operation and maintenance of high field NMR spectrometers (600 and 800 MHz) and cryogenic probe systems is required, and knowledge of hardware and Unix-based software maintenance is essential. Familiarity with Varian systems and methods of NMR data analysis through structure calculation and validation stages are also desirable but not necessary.

Applicants should submit a brief statement of interest along with curriculum vitae and should arrange for three letters of recommendation to be sent to: **Prof. James P. Reilly, Chair of Search Committee, Department of Chemistry, Indiana University, 800 E. Kirkwood Avenue, Bloomington, IN 47405. Fax: 812-856-5050; e-mail: chemchair@indiana.edu.** Applications received by December 15, 2009, will be assured of consideration. *Indiana University is an Equal Opportunity Affirmative Action Employer.*

PROTEOMICS LOYOLA UNIVERSITY CHICAGO

The Departments of Biology and Chemistry at Loyola University Chicago invite applications for a joint position in proteomics at the **ASSISTANT PROFESSOR** level, beginning in August 2010. Applicants from all areas of proteomics will be considered, but preference will be given to candidates whose research focus would engender collaboration with existing faculty. A Ph.D. in biology (or appropriate subdiscipline), chemistry, or biochemistry is required, as is relevant postdoctoral experience. The successful candidate is expected to maintain an internationally competitive, externally funded research program involving undergraduate, M.S., and Ph.D. students, and to participate in the ongoing development of interdisciplinary and advanced degree programs. Teaching responsibilities include proteomics, molecular biology, and a graduate level seminar in the appointee's area of expertise. The candidate will join the 20-member faculty in the interdisciplinary Bioinformatics B.S. degree program that was launched in 2005. Applicants should complete the online application in full by going to [website: http://www.careers.luc.edu](http://www.careers.luc.edu). Pending final funding, review of applications will begin on December 15, 2009, and continue until the position is filled. Information about the Biology and Chemistry Departments and the Bioinformatics Program are available at [websites: http://www.luc.edu/biology](http://www.luc.edu/biology), <http://www.luc.edu/chemistry>, and <http://www.luc.edu/bioinformatics/>. *LUC, Chicago's Jesuit Catholic University, is an Equal Opportunity/Affirmative Action Employer with a strong commitment to diversifying its faculty. Applications from women and minority candidates are especially encouraged.*

RESEARCH ASSOCIATE position available at VA New York Medical Center for an **ELECTROPHYSIOLOGIST**. Candidate must have extensive experience with all biochemical/molecular cardiology and patch clamp techniques. Additional experience with cell transfection, confocal and calcium imaging is desired. Send statement of interest, curriculum vitae, and three letters of reference to: **Dr. Mohamed Boutjdir, e-mail: mohamed.boutjdir@va.gov.**

POSITIONS OPEN



POSTDOCTORAL FELLOWS

The laboratory of **Donal G. Sinex** (e-mail: don.sinex@usu.edu) at Utah State University seeks two Postdoctoral Fellows to participate in computational and psychophysical studies of the processing of complex sounds and speech. The emphasis will be on understanding the perception of competing sounds, including the identification of speech in the presence of noise. Both positions are available immediately and will be supported by American Recovery and Reinvestment Act funding for two years. Ph.D. or equivalent in psychology, neuroscience, engineering, or a related field is required. See [website: http://jobs.usu.edu](http://jobs.usu.edu) (requisition #051877 and #051878) for full job descriptions and to apply online. *Affirmative Action/Equal Opportunity Employer.*

POSTDOCTORAL POSITIONS in BIOMOLECULAR NETWORKS

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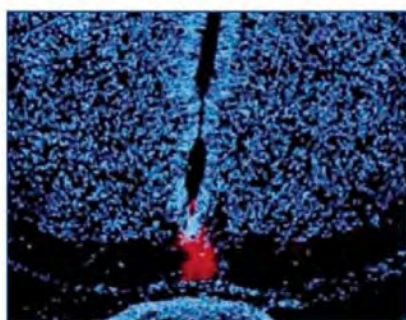
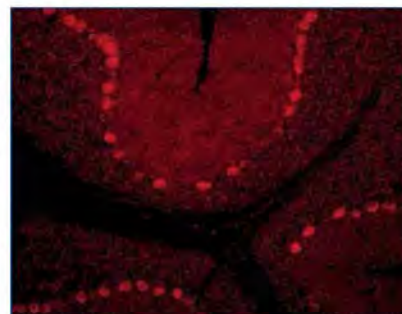
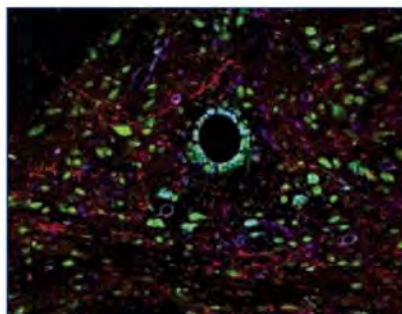
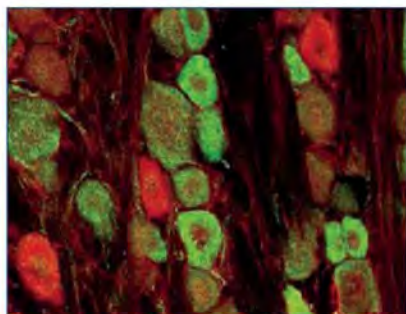
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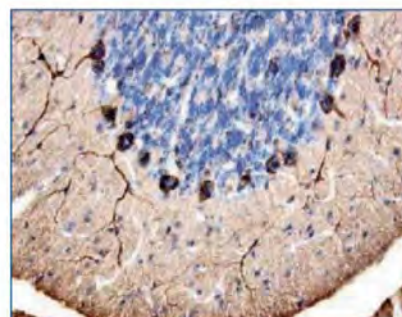
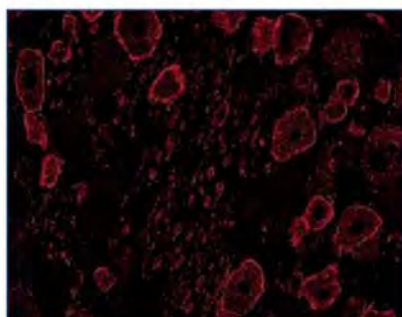
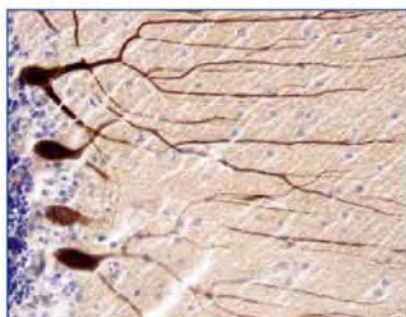
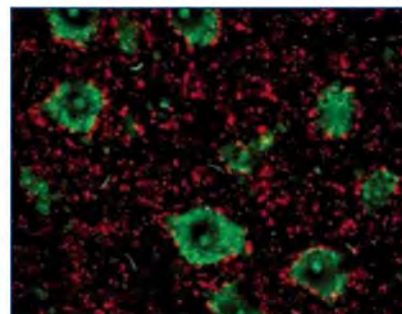
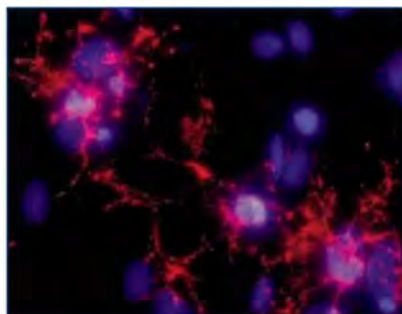
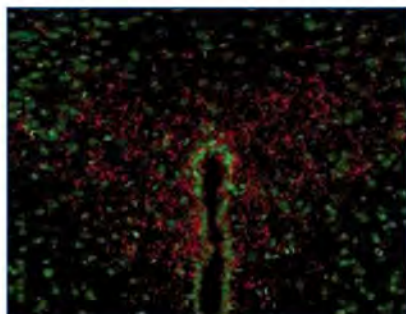
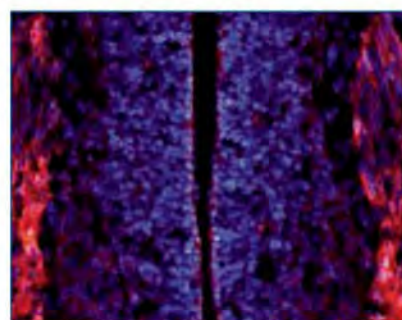
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